

THE
AMERICAN JOURNAL
OF
PHYSIOLOGY

VOLUME 117

BALTIMORE, MD.
1936

CONTENTS

No. 1. SEPTEMBER, 1936

The Rôle of the Thyroid in the Calorigenic Action of Vitamin D. <i>H. Deutsch, C. I. Reed and H. C. Struck</i>	1
The Effect of Experimental Hyperthyroidism on Carbohydrate Metabolism. I. <i>Arthur Mirsky and R. H. Broh-Kahn</i>	6
A Study of the Blood Sugar of the Adrenalectomized Dog. <i>W. M. Parkins, H. W. Hays and W. W. Swingle</i>	13
Depression in Order of Frequency of the Electrical Cochlear Response of Cats. <i>Edmund P. Fowler, Jr. and T. W. Forbes</i>	24
A Study of the Average Temperature of the Tissues, of the Exchanges of Heat and Vasomotor Responses in Man by Means of a Bath Calorimeter. <i>A. C. Burton and H. C. Bazett</i>	36
The Production of Sympathin in Response to Physiological Stimuli in the Unanesthetized Animal. <i>Philip P. Partington</i>	55
Dietary and Hematologic Studies after Gastrectomy in the Rat. <i>Robert A. Bussabarger and Frederic T. Jung</i>	59
Augmentation of the Gonad Stimulating Action of Pituitary Extracts by Inorganic Substances, Particularly Copper Salts. <i>H. L. Fevold, F. L. Hisaw and R. Greep</i>	68
The Effectiveness of Carbon Dioxide in Combating the Changes in Visual Intensity Discrimination Produced by Oxygen Deficiency. <i>E. Gellhorn</i>	75
The Rôle of the Duodenal Secretions in the Prevention of Experimental Jejunal Ulcer. <i>Charles M. Wilhelmj, F. T. O'Brien, H. H. McCarthy and Frederick C. Hill</i>	79
The Endometrial Vascular Bed in Relation to Rhythmic Uterine Motility, with a Consideration of the Functions of the Intermittent Contractions of Oestrus. <i>Joseph Fagin and Samuel R. M. Reynolds</i>	86
The Effect of Certain Sulfur Compounds on the Coagulation of Blood. <i>J. H. Sterner and Grace Medes</i>	92
A Comparison of Three Methods of Measuring Plasma Dilution after Intravenous Saline Injection into Normal Anesthetized and Functionally Eviscerated Dogs. <i>Allan Hemingway, Dean A. Collins and F. Bernhart</i>	102
The Specific Gravity of the Blood of Pigeons in the Quiet State and During Emotional Excitement. <i>L. B. Nice and David Fishman</i>	111
Action and Excitability in Mammalian A. Fibers. <i>Herbert S. Gasser and Harry Grundfest</i>	113
The Utilization of Fructose in the Mammalian Organism as Shown by Experiments on Hepatectomized and Eviscerated Preparations. <i>Jean P. Griffiths and E. T. Waters</i>	134
In Vitro Action of Crystalline Vitamin B ₁ on Pyruvic Acid Metabolism in Tissues from Polyneuritic Chicks. <i>W. C. Sherman and C. A. Elvehjem</i>	142
A Study of Anaerobic Glycolysis in Tissues from Polyneuritic Chicks. The Negative Action of Vitamin B ₁ . <i>W. C. Sherman and C. A. Elvehjem</i>	151

Further Studies on the Effects of NaF Administration upon the Basal Metabolic Rate of Experimental Animals. <i>Paul H. Phillips</i>	155
The Relation of Pancreatic Juice to Pancreatic Diabetes. <i>Herman P. Harms, John Van Prohaska and Lester R. Dragstedt</i>	160
The Relation of Pancreatic Juice to the Fatty Infiltration and Degeneration of the Liver in the Depancreatized Dog. <i>John Van Prohaska, Lester R. Dragstedt and Herman P. Harms</i>	166
Observations on a Substance in Pancreas (A Fat Metabolizing Hormone) Which Permits Survival and Prevents Liver Changes in Depancreatized Dogs. <i>Lester R. Dragstedt, John Van Prohaska and Herman P. Harms</i>	175
Histone Combinations of the Protein Hormones. <i>Fritz Bischoff</i>	182

No. 2. OCTOBER, 1936

Skin Potential and Impedance Responses with Recurring Shock Stimulation. <i>T. W. Forbes</i>	189
Survival of the Adrenalectomized Nephrectomized Rat. <i>Dwight J. Ingle and Edward C. Kendall</i>	200
Effect of Epinephrine on Glucose Excretion in Fasted Depancreatized Dogs. <i>W. H. Bachrach, W. B. Bradley and A. C. Ivy</i>	203
Glomerular Filtration and Urea Excretion in Relation to Urine Flow in the Dog. <i>James A. Shannon</i>	206
The Effect of Acute Hemorrhage on the Emptying Time of the Stomach. <i>Edward J. Van Liere, Clark K. Sleeth and David Northup</i>	226
Total Plasmapheresis. <i>John B. Stanbury, Edna Warweg and William R. Amberson, with the technical assistance of Verda I. McLendon</i>	230
The Activity of the Cardiac Sympathetic Centers. <i>D. W. Bronk, L. K. Ferguson, R. Margaria and D. Y. Solandt</i>	237
A Possible Rôle of the Eosinophil Leucocytes in the Endocrine Complex of the Female Rat. <i>C. P. Kraatz</i>	250
A Study of the Speed of Absorption Following the Ingestion of Glucose and of Sucrose. <i>Alice C. Roberts</i>	257
Electrical Stimulation of the Interior of the Cerebellum in the Decerebrate Cat. <i>W. K. Hare, H. W. Magoun and S. W. Ranson</i>	261
The Spinal Path for Responses to Cerebellar Stimulation. <i>E. H. Ingersoll, H. W. Magoun and S. W. Ranson</i>	267
Blood Flow in the Circumflex Branch of the Left Coronary Artery of the Intact Dog. <i>Hiram E. Essex, J. F. Herrick, Edward J. Baldes and Frank C. Mann</i>	271
Carotene and Associated Pigments in Medullated Nerve. <i>John Paul Bartz and Francis O. Schmitt</i>	280
Germinal Response (In Male Mice) to Environmental Conditions. <i>Cordelia L. Ogle</i>	285
Components of the Electrical Response of the Optic Cortex of the Rabbit. <i>G. H. Bishop and James O'Leary</i>	292
Absorption of Sodium Chloride from the Small Intestine at Various Degrees of Anoxemia. <i>Edward J. Van Liere and Clark K. Sleeth</i>	309
Transplantation of Sino-Atrium to Conus in the Embryonic Heart in Vitro. <i>George H. Paff</i>	313
Increased Water Exchange following Eck Fistula In Dogs. <i>Lathan A. Crandall, Jr. and George M. Roberts</i>	318
The Glucose Utilization of Phloridzinised Dogs after Hepatectomy. <i>D. R. Drury, H. C. Bergman and Paul O. Greeley</i>	323
Observations on the Blood Flow and Gaseous Metabolism of the Liver of Unanesthetized Dogs. <i>Alfred Blalock and Morton F. Mason</i>	328

The Distribution of Glucose in Blood. <i>Isaac Neuwirth</i>	335
A Comparison of the Electrogram of the Optic Cortex with that of the Retina. <i>S. Howard Bartley</i>	338
Respiratory Reactions upon Vertical Movements. <i>E. A. Spiegel</i>	349
Temporal Summation in Peripheral Nerve Fibers. <i>E. A. Blair and Joseph Erlanger</i>	355
Control of Urine Formation in the Frog by the Renal Circulation. <i>E. F. Adolph</i> ..	366

No. 3. NOVEMBER, 1936

Ovarian Hormone Threshold for Experimental Menstruation in Monkeys. <i>Edgar Allen, A. W. Diddle, T. H. Burford and W. U. Gardner</i>	381
The Site of Action of Botulinus Toxin. <i>George H. Bishop and Jacques J. Bronfenbrenner</i>	393
The Tetany of Oestrus in the Parathyroidectomized Dog. <i>Everett I. Evans, S. Szurek and R. Kern</i>	405
Cardiovascular Reactions Induced by Electrical Stimulation of the Cerebral Cortex. <i>Ebbe C. Hoff and Harold D. Green</i>	411
Structural and Functional Organization of the Central Mechanism Controlling Breathing. <i>Robert Gesell, John Bricker and Conway Magee</i>	423
The Effect of Intravenous Administration of Protamine Insulin. <i>Bernard B. Longwell and Abe Ravin</i>	453
The Mechanism of the Inhibitory Action of Vasodilator Nerves. <i>Emil Bozler</i> ...	457
Effects of Anatomical Separation of the Hypophysis from the Hypothalamus in the Dog. <i>Allen D. Keller, William Noble and J. William Hamilton, Jr.</i>	467
The Measurement of Serum Volume. <i>F. William Sunderman and J. H. Austin</i> ..	474
The Effect of Lactation and Exercise on the Rate of Involution of the Uterus in the Rat. <i>Elizabeth Abbott and A. C. Ivy</i>	487
The Crossed Respiratory Impulses to the Phrenic. <i>A. Rosenblueth and T. Ortiz</i> ..	495
Functional Behavior of Coeliac Ganglion Cells of the Rabbit. <i>E. H. Ingersoll</i>	514
Thyrotropic Effect of Pituitaries from Cretin Rats. <i>Isolde T. Zeckwer</i>	518
The Origin of Fecal Fat in the Absence of Bile, Studied with Deuterium as an Indicator. <i>Arthur Shapiro, Harry Koster, D. Rittenberg and Rudolf Schoenheimer</i>	525
A Comparison of the Chemical Composition of Stimulated and Resting Saliva of Caries-free and Caries-susceptible Children. <i>Julius White and Russell W. Bunting</i>	529
Gastric Acidity Following Partial Gastrectomy and Vagotomy. <i>Charles M. Wilhelmj, H. H. McCarthy and Frederick C. Hill</i>	533
The Effect of Acetylcholine and Other Constituents of the Suprarenal Gland upon Blood Sugar and Amino Acids. <i>Burt Lincoln Davis, Jr. and J. Murray Luck</i> ..	542
A Further Study of the Relation of the Adrenal Cortex to Vitamin C. <i>Julia E. Lockwood, Donald R. Swan and Frank A. Hartman</i>	553
The Effect of Occlusion of the Outflow of Prostatic Secretion on the Prostate Gland. <i>James I. Farrell and Yale Lyman</i>	559
The Role of the Anterior Hypothalamus in Temperature Regulation. <i>R. S. Teague and S. W. Ranson</i>	562
Emotional Leucopenia in Rabbits. <i>L. B. Nice and H. L. Katz</i>	571

No. 4. DECEMBER, 1936

The Respiratory Responses of Pre-adolescent Boys to Muscular Activity. <i>Edward C. Schneider and C. B. Crampton</i>	577
An Experimental Analysis of Coagulant Activation. <i>John H. Ferguson</i>	587

The Action of a Single Vagal Volley on the Heart of the Eel and the Turtle. <i>Ernst Fischer</i>	596
The Extinction of Startle Responses and Spinal Reflexes in the White Rat. <i>C. Ladd Prosser and Walter S. Hunter</i>	609
Spinal Vasomotor Reflexes Associated with Variations in Blood Pressure. <i>C. Heymans, J. J. Bouckaert, Sidney Farber and F. Y. Hsu</i>	619
A Study of "Simple Disuse Atrophy" in the Monkey. <i>Herman Chor and Ralph E. Dolkart</i>	626
The Effect of Methylene Blue, Cystine and Cysteine on the Metabolism of the Intact Animal. <i>Walter Goldfarb, Joseph F. Fazekas and Harold E. Himwich</i>	631
Strychnine and the Chronaxie. <i>P. K. Knoefel</i>	638
The Relationship of the Synthetic Male Hormone, Androstendion, to the Protein and Energy Metabolism of Castrate Dogs, and the Protein Metabolism of a Normal Dog. <i>Charles D. Kochakian and John R. Murlin</i>	642
Urea Clearance and Proteinuria During Exercise. <i>Arthur B. Light and Clark R. Warren</i>	658
The Concentration of Nucleated Cells in the Bone Marrow of the Albino Rat. <i>George E. Farrar, Jr.</i>	662
The Blood Clearance and Renal Excretion of Bile Acids Following the Intravenous Injection of Cholic and Desoxycholic Acids. <i>S. S. Lichtman</i>	665
Vaginal and Uterine Grafts in the Rat as Indicators of the Production of Oestrin. <i>Carroll A. Pfeiffer</i>	672
The Bioassay of Adrenal Cortical Extracts. A Direct Comparison of Rat and Dog Units <i>George F. Cartland and Marvin H. Kuizenga</i>	678
The Potential Analysis of a Pacemaker Mechanism in <i>Limulus Polyphemus</i> . <i>Peter Heinbecker</i>	686
Observations on the Response of the Spleen to the Intravenous Injection of Certain Secretin Preparations, Acetyl Choline and Histamine. <i>John Ferguson, A. C. Ivy and Harry Greengard</i>	701
Study of Depth Temperatures in Artificial Fevers and Cooling Air Chambers with Especial Reference to Cooling Effect of the Circulating Blood. <i>John J. Sampson</i>	708
Index	717

THE AMERICAN JOURNAL OF PHYSIOLOGY

VOL. 117

SEPTEMBER 1, 1936

No. 1

THE RÔLE OF THE THYROID IN THE CALORIGENIC ACTION OF VITAMIN D¹

H. DEUTSCH, C. I. REED AND H. C. STRUCK

From the Department of Physiology, College of Medicine, University of Illinois, Chicago

Received for publication May 18, 1936

It has been shown that vitamin D in massive, but subtoxic, doses will enormously increase the metabolic rate of normal dogs and rats (1, 2). Landelius and Ljungkvist (6) have recently confirmed the earlier observations of Seel (7) that vitamin D will restore the rate of oxygen consumption to normal when it has been decreased in rickets. Goormaghtigh and Handovsky (3) have presented evidence of a thyrotropic influence of vitamin D and Gelfan (4) has shown that isolated muscles of frogs treated with vitamin D utilize more oxygen than do those from normal frogs. These latter experiments are unique in another respect, i.e., the demonstration of a response of a cold blooded form to vitamin D.

That the calorogenic effect is not due to an action on the parathyroids is indicated by the report of Reed, Steck and Miller (5) that parathyroid extract was wholly ineffective as a calorogenic agent in normal dogs.

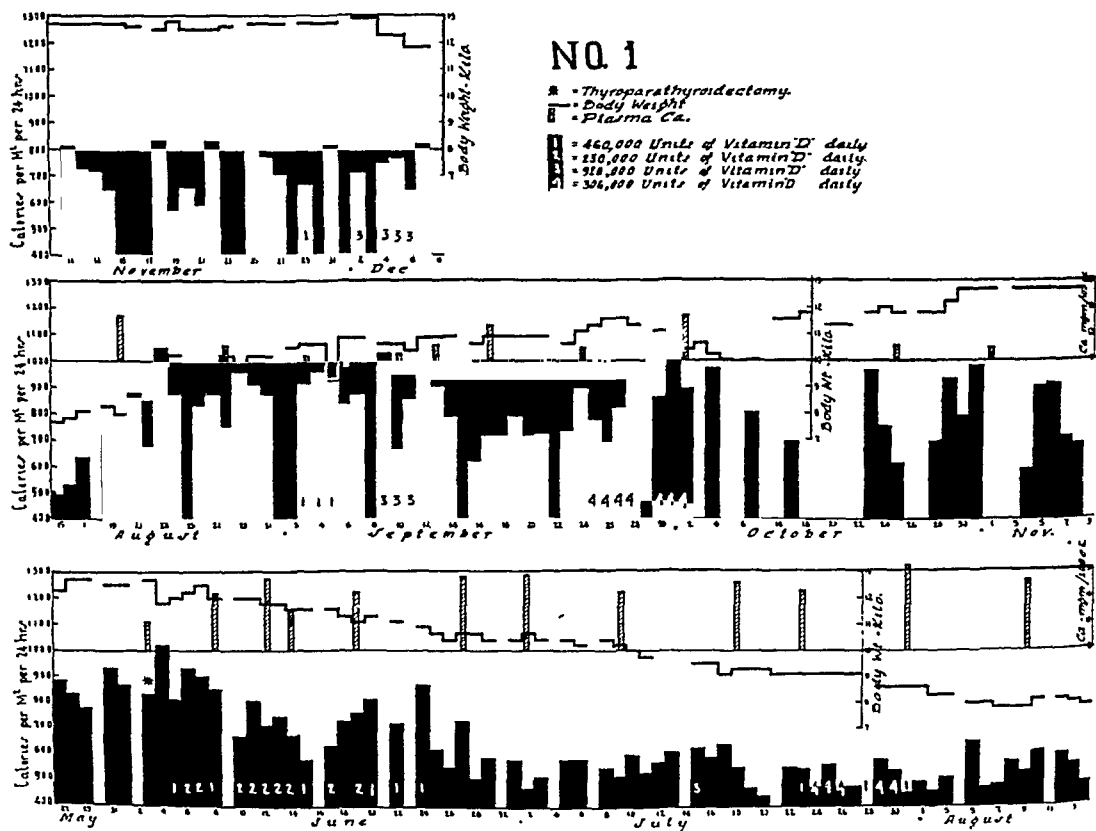
It is the purpose of this paper to report experiments on two dogs which demonstrate the importance of the thyroid in this reaction. The basal metabolic rate was determined on two normal dogs over a preliminary control period after which both were subjected to complete thyroparathyroidectomy. At autopsy no accessory thyroid tissue was found. It is, of course, possible that there may still have been microscopic rests of accessory tissue. But in any case, the total amount of thyroid tissue was *reduced* to a minimum. The results are described in the protocols and illustrated in the figure.

¹The expenses of this investigation were in part defrayed by grants from the Wisconsin Alumni Research Foundation and from Mead Johnson and Company. The vitamin D in the form of viosterol (1,000,000 units per gram) was supplied by the latter.

A preliminary report of this work was read before the American Physiological Society, March 27, 1936.

Dog 1, young adult, male, 12.5 kilos (fig. 1).

The average metabolic rate for a period of 3 weeks before operation was 850 calories per square meter per 24 hours. Only 6 preoperative determinations are shown on the chart, but the variations before that were comparable to those charted. On June 3 a total thyroidectomy was done. No particular effort was made to remove the parathyroids, although it was ascertained later that the main bodies were all removed. No accessory thyroid tissue was found at autopsy. The concentration of total blood calcium was 11.11 mgm. per 100 cc. of heparinized plasma before the operation. There was a mild postoperative caloric reaction but in general the metabolic rate decreased progressively through June and July, approximately parallel with the decline in body weight to 7.75 kgm. on August 15. A diet of raw



beef was now substituted. With the prompt recovery of weight the metabolic rate increased to a normal level.

Oral administration of vitamin D was done in 5 periods, as follows:

	• units
May 5 to June 14.....	4,830,000
July 15 to August 31.....	3,830,000
September 3 to September 11.....	4,140,000
September 25 to October 1.....	1,142,000
November 29 to December 6.....	3,220,000

During the period of weight decline to August 15 the metabolic rate fluctuated

between 500 and 600 calories/m²./24 hours. The average from August 23 to September 3 was 893 cal. During the third period of vitamin D administration and the three succeeding days, the average was 875 cal.; from September 16 to 25, the average was 738; from September 25 to October 7, the average was 877.

After the operation the blood calcium remained high (14-20 mgm./100 cc.) until August 28. Thereafter it fluctuated between 7.8 and 15 mgm.

Dog 2, young adult male, 12.5 kilos.

The preoperative metabolic rate over 18 days averaged 930 cal./m²./24 hours. June 16, complete thyroidectomy. There was a postoperative increase in the metabolic rate for 3 days, then a definite decline to 620 calories on August 15, at which time the weight was 9.5 kilos. On a raw meat diet the weight increased to 12 kilos by September 4. The total administration of vitamin for each of 4 periods was as follows:

	<i>units</i>
June 18 to 28.....	2,070,000 (no calorigenic effect)
July 15 to 31.....	4,444,000 (no calorigenic effect)
September 10 to 11.....	1,840,000 (no calorigenic effect)
September 25 to October 1.....	2,142,000 (see text)

During the period of weight gain from August 16 to September 4 the average metabolic rate was 880, but the fluctuations were very wide. From September 5 to 15 inclusive the average was 810 cal. During the last period of vitamin D administration and the subsequent 4 days the average rate was 842 cal. The average rate from October 28 to December 15 inclusive was 800 cal.

In the experiments on normal dogs the oral administration of amounts of vitamin D such as were given to both of these animals produced definitely, sustained augmentation of the metabolic rate within 3 to 5 days. The only suggestion of a calorigenic effect in either of these animals was seen in no. 1 on October 1 and in 2 during the week immediately following the fourth period of vitamin D administration when the rate was slightly higher than immediately before or after. In neither instance, however, were the effects anything like those seen in normal dogs with comparable doses of vitamin D.

The spontaneous variation occurring in these animals was very striking. It was much more difficult to get consistent results than in any normal dog we have ever trained. It would seem to be a valid assumption that this instability is a manifestation of the combination of the hypothyroid and hypoparathyroid states. The restoration of the metabolic rate concurrently with weight gain was a result that might have been predicted but was unexpected.

The marked variations occurring in no. 2 through August were correlated with a state of extreme nervous irritability that did not appear to be a manifestation of incipient tetany. While it is not impossible for tetany to occur with a high concentration of blood calcium such a result is unusual; especially when it is considered that at no time did either of these animals display any tetanic symptoms.

However, granting that some of the temporary fluctuations were due to the calorogenic effect of vitamin D, there certainly was no such response as occurred in dogs with normal thyroids. That no. 1 became intoxicated to a mild degree by the 4th period of vitamin D administration is indicated by the sharp decline in weight from September 27 to October 7. Past experience would lead one to expect that such intoxication would give a still greater augmentation of metabolism.

If one assumes that in the two instances cited there was a mild augmentation of metabolism, the mechanism by which it was produced must come in for consideration. Since no accessory thyroid tissue was found the total amount present must be so small that it seems unlikely that it could produce any effect.

The work of Gelfan suggests a possible effect on peripheral tissues. The effect was not unlike that observed in isolated muscle from thyroxinized warm blooded forms. But since there is no agreement that thyroxin produces such a calorogenic influence in cold blooded forms it cannot, at present, be assumed that the effects he obtained were due to thyroid stimulation. In that case the only other apparent explanation would be that the vitamin stimulates metabolism in peripheral tissues. Until some method is evolved for applying vitamin D directly to isolated peripheral tissues this point must remain unsettled.

That the calorogenic effect is not correlated with hypercalcemia was pointed out in the earlier work (1). This is still more definitely emphasized in these experiments when one considers that in both dogs the blood calcium was very high during the entire extent of the period of low metabolic rate.

Dog 1, after recovery of the original weight remained practically stationary while dog 2 had a second period of weight gain beginning September 16 and ultimately reaching 17.25 kgm. on November 15. However, there was no augmentation of metabolism correlated with this gain.

Whether the thyreotropic effect is due to direct action of vitamin D on the thyroid or to indirect action through the anterior pituitary is now under investigation.

While it has been maintained and is still maintained by some investigators that the administration of massive dosage of vitamin D has a direct effect on the parathyroids, it is clear from this and the preceding investigations that at least this particular effect of vitamin D does not involve the parathyroids. In fact, Steck, Reed and Miller (5) have shown that parathyroid extract tends to lower the metabolic rate, if anything. Furthermore, in one of their animals, long continued administration of parathyroid extract did not desensitize the animal to the subsequent calorogenic action of vitamin D.

Most investigators have assumed a rather limited physiological action of vitamin D. It seems probable, however, that its action on calcium-phosphorus metabolism is only one of many effects. We have considerable data supporting this view which will be presented later.

SUMMARY AND CONCLUSIONS

1. After complete thyroparathyroidectomy in two dogs there was a pronounced decrease in the metabolic rate correlated with loss in weight. With recovery of weight, the metabolic rate also was restored to nearly the original level.

2. Large doses of vitamin D produced no marked augmentation in the metabolic rate comparable to that produced in normal dogs.

3. This effect of vitamin D is not due to an action on the parathyroids.

REFERENCES

- (1) REED, C. I., E. A. THACKER, L. M. DILLMAN AND J. W. WELCH. *J. Nutrition* **6**: 355, 1933.
- (2) REED, C. I. *Proc. Soc. Exper. Biol. Med.* **32**: 274, 1934.
- (3) GOORMAGHTIGH, N. AND H. HANDOVSKY. *Compt. Rend. Soc. Biol.* **118**: 1616, 1935.
- (4) GELFAN, S. *This Journal* **113**: 464, 1935.
- (5) STECK, I. E., C. I. REED AND D. S. MILLER. *This Journal* **110**: 1, 1934.
- (6) LANDELIUS, E. AND G. LJUNGKOIST. *Skand. Arch. f. Physiol.* **68**: 252, 1934.
- (7) SEEL, H. *Arch. f. exper. Path. u. Pharmakol.* **140**: 194, 1929.

THE EFFECT OF EXPERIMENTAL HYPERTHYROIDISM ON CARBOHYDRATE METABOLISM¹

I. ARTHUR MIRSKY AND R. H. BROH-KAHN

From the Department of Metabolism and Endocrinology, Institute for Medical Research, Jewish Hospital, Cincinnati

Received for publication May 21, 1936

Although it is acknowledged by many that hyperthyroidism is associated with a profound disturbance in carbohydrate metabolism, the nature of the derangement is obscure. The associated syndrome of glycosuria, ketosis, low respiratory quotient and abnormal dextrose tolerance, as judged by the dextrose tolerance curve, resembles in many respects the syndrome observed in "hunger diabetes," pancreatic diabetes, and phlorhizin diabetes. This similarity is further emphasized by the fact that when hyperthyroidism develops in the course of diabetes mellitus, the diabetic condition is aggravated.

Such factors have led some investigators to assume that hyperthyroidism is associated with a disturbance either of the insulinogenic mechanism or the peripheral action of insulin. Others attribute the hyperthyroid syndrome to a relative diminution of carbohydrate oxidation subsequent to the depletion of glycogen stores. It is now well established that in animals the feeding of thyroid is followed by such a depletion (1). Cramer and Krause (2) interpreted this to mean that the storage of glycogen was defective in consequence of a decreased oxidation of carbohydrate. However, Sanger and Hun (3) after studying simultaneous curves of the respiratory exchange and blood sugar in a large series of patients concluded that there was no defect in the oxidation but rather an abnormality in the mobilization of glucose, namely, a defect in the mechanism by which glycogen is stored. This is in agreement with the observations of Cramer and M'Call (4) who found that thyroid-fed rats suffered not a decrease but an increase in the oxidation of carbohydrate. However, they attributed the changes to a primary defect in the glycogenic mechanism and a secondary increase in the oxidation of carbohydrate. From a series of respiratory studies on human subjects, Richardson, Levine and DuBois (5) and Johnston (6) concluded that there was no disturbance in the glycogen-storing mechanism but that the glycogenolytic mechanism was very unstable.

¹ Aided by the David May Memorial Fund.

In view of the fact that there are no direct observations on the utilization of carbohydrate in experimentally produced hyperthyroidism, we studied the rate of disappearance of glucose from the blood of eviscerated, hyperthyroid and normal rabbits. If either an increased rate of hepatic glycogenolysis "per se" or a decreased hepatic glycogenesis is the primary metabolic disturbance responsible for the diminution of glycogen observed in thyroid-fed animals, the rate of glucose removal by the muscles from the blood would be the same in both the normal and the hyperthyroid, eviscerated rabbit since the liver is not present to influence the blood sugar nor can tissue glycolysis be a significant factor (7). On the other hand, if an acceleration of carbohydrate utilization by the muscles is the primary disturbance, the rate of disappearance should be greater in the hyperthyroid than in the normal, eviscerated animal. By this means it should be possible to determine whether the utilization of carbohydrate or some derangement in the mechanism of glycogen storage is the primary disturbance in the hyperthyroid animal.

METHOD. Male New Zealand rabbits weighing from 1.5 to 2.0 kgm. in body weight were used in this study. Before the preliminary twenty-four hour fast, the animals were maintained on a commercial diet (Purina rabbit chow). Nembutal anesthesia was employed throughout all experiments in order to allow for the operative procedures and for the withdrawal of blood from the exposed femoral artery.

Evisceration was performed by a one-stage operation somewhat similar to that described by McMaster and Drury (8) for partial hepatectomy. The abdomen was opened and the intestines retracted to the right, exposing the aorta and inferior vena cava. The inferior mesenteric artery, the superior mesenteric artery and the coeliac axis were exposed and cut between ligatures. The gastro-intestinal tract was removed after cutting the rectum, esophagus and portal vein between ligatures. The arrangement of the rabbit liver into almost separate lobes permits the removal of practically the whole liver by simple ligation and excision. This is particularly simple in young rabbits where the lobes tend to be more distinct than in older animals. The fragments of liver that remain after this operation can be crushed between the fingers. The kidneys were removed after ligation of the hilus. The complete evisceration in this manner can be easily performed in about fifteen minutes.

In order to ascertain the completeness of the hepatectomy, we performed several eviscerations in rabbits employing the method of Markowitz, Yater and Burrows (9) where the inferior vena cava was replaced with a pyrex cannula. In several other instances, the two-stage operation of Drury (10) was performed. Comparison of our results with these procedures revealed no significant differences in the rate at which sugar was removed from the blood, and, hence, we subsequently confined ourselves to the above described procedure.

Hyperthyroidism was produced by the daily, oral administration of ten grain tablets of desiccated thyroid until a twenty to forty per cent loss of body weight occurred. This usually took from six to seven days.

Arterial blood samples were drawn immediately after the completion of the evisceration and at half-hourly intervals thereafter for one to two hours and the glucose content determined by the Somogyi modification of the Shaffer-Hartman method. In the majority of experiments with the hyperthyroid rabbits, glucose was administered at the end of the first hour, and, after a fifteen minute interval, the blood sugar decrement was studied for another hour. In all instances, the rate of glucose removal

TABLE 1

RABBIT NUMBER	DISAPPEARANCE OF BLOOD SUGAR FOLLOWING EVisCERATION		
	Immediate	30 minutes	60 minutes
	<i>mgm. per cent</i>	<i>mgm. per cent</i>	<i>mgm. per cent</i>
Control 1.....	50		25
Control 2.....	54		28
Control 3.....	59		37
Control 4.....	80	64	55
Control 5.....	85	65	59
Control 6.....	92	76	67
Control 7.....	101	83	74
Control 8.....	105		69
Hyperthyroid 1.....	48	15	
Hyperthyroid 2.....	78	38	10
Hyperthyroid 3.....	86	53	32
Hyperthyroid 4.....	87	56	18
Hyperthyroid 5.....	97	54	16
Hyperthyroid 6.....	104	62	25
Hyperthyroid 7.....	118	76	32
Hyperthyroid 8.....	87	51	29

during the second hour was, within the limits of experimental error, the same as that during the first hour.

RESULTS. Some of the results are detailed in table 1. Analysis of the control group reveals that the blood sugar dropped from 22 to 26 mgm. per cent in one hour whereas that of the hyperthyroid group fell from 54 to 86 mgm. per cent in the same interval of time. The average decrease in the blood sugar per hour was 26.5 mgm. per cent, with a standard deviation of ± 3.85 mgm. for the control group, and 70.7 mgm. per cent, with a standard deviation of ± 11.09 mgm. for the hyperthyroid group. Because of the difference in blood sugar level and the fact that the fall of the blood sugar was determined by the initial level, we computed the

rate of disappearance as the *per cent drop from the initial level*. The average sugar disappearance in one hour was 35.6 per cent for the control group and 75.5 per cent for the hyperthyroid group (fig. 1). In some instances, the rate of glucose disappearance from the blood of the hyperthyroid rabbits was so rapid that we obtained very low initial blood sugar levels and it was expedient to administer glucose within one hour to prevent the death of the animal.

DISCUSSION. It is obvious from the above data that the extrahepatic tissues of the hyperthyroid rabbit remove glucose from the blood at a much greater rate than do those of the normal animal. Since the observations of Andrus and McEachern (7) indicate that the tissues of the hyperthyroid animal do not have a significantly increased glycolysis, it becomes probable that the increased rate of blood glucose removal which such animals exhibit is due to an increase in carbohydrate utilization. Furthermore, since the muscles of thyroid-fed animals are characterized by a marked depletion of glycogen, the glucose is probably used for purposes other than storage. Thus we are led to conclude that there is a marked acceleration in carbohydrate oxidation in the tissues of the hyperthyroid animal, and that the increased oxygen utilization of the intact hyperthyroid animal or of isolated tissues from such an animal is due to this increased rate of carbohydrate oxidation.

These observations exclude the possibility that the primary disturbance of the carbohydrate metabolism in hyperthyroidism is some defect in the glycogen storing mechanism of the liver, or a diminution of carbohydrate utilization by the extrahepatic tissues. The decreased liver glycogen content that is commonly observed in thyroid-fed animals is probably a secondary phenomenon consequent to 1, an increased utilization of carbohydrate by the liver itself with the onset of hyperthyroidism, and 2, an increased rate of removal of glucose from the liver by the extrahepatic tissues (glycogenolysis). A similar increase in hepatic glycogenolysis is known to occur in pancreatic and phlorhizin diabetes. In the former, Major and Mann (11) have demonstrated that glycogen can be synthe-

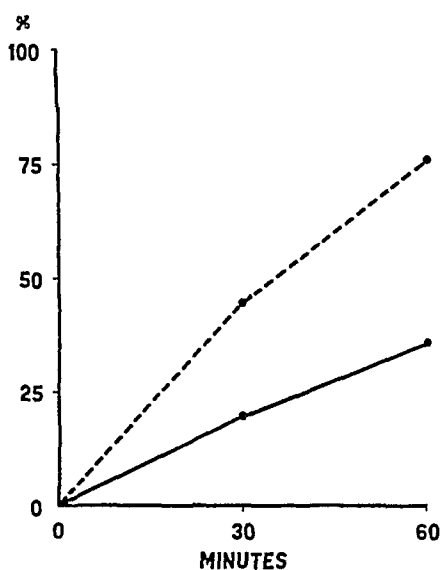


Fig. 1. Graphic comparison of the rates of disappearance of the blood sugar after complete evisceration in normal (solid line) and hyperthyroid (broken line) rabbits. Each curve represents the average per cent drop from the initial blood sugar level.

sized in the liver but that it cannot be retained unless insulin is administered, indicating that in this condition there is an inability of the cells to hold glycogen, so that the rate of glycogenolysis exceeds the rate of glycogenesis. In phlorhizin diabetes, where the renal threshold for glucose is lowered, an accelerated hepatic glycogenolysis occurs in response to the hypoglycemia which results from the loss of glucose via the kidneys. Thus

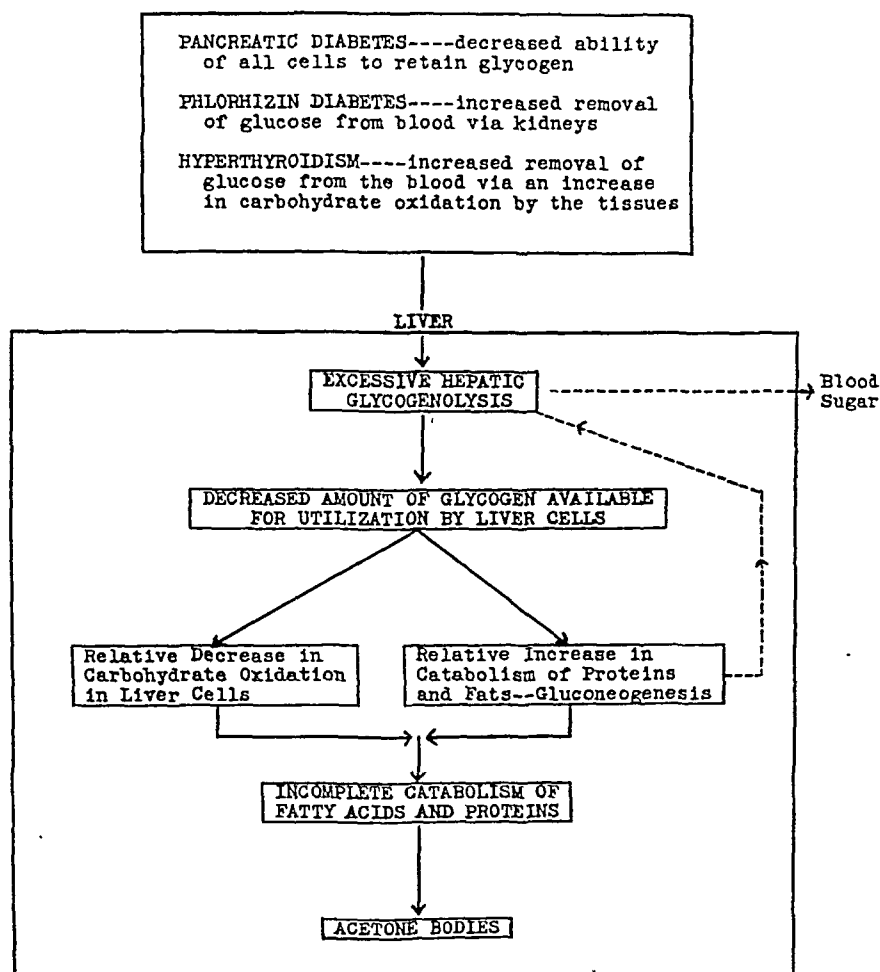


Fig. 2

in these three conditions an accelerated hepatic glycogenolysis is a common factor, being primary in pancreatic diabetes and secondary in phlorhizin diabetes and in hyperthyroidism.

In hyperthyroidism, as in the other conditions discussed above, ketosis is a frequent occurrence especially after a relatively short period of starvation. This cannot be attributed to a decrease in the utilization of carbohydrates by the extrahepatic tissues since our data indicate that the re-

verse actually occurs in hyperthyroidism. Furthermore, it is obvious from previously reported studies that the muscles do not contribute to the accumulation of ketones in the blood and that the liver is probably the only site of ketone body formation (12). The fact that starvation "per se" ("hunger diabetes") is not associated with a decrease in the utilization of glucose by the extrahepatic tissues lends support to this conclusion (13). These observations are in accord with a tentative hypothesis which we have previously presented in explanation of the mechanism responsible for ketone formation (12, 14). According to this hypothesis (fig. 2), a decrease in liver glycogen is tantamount to a decrease in the amount of carbohydrate available for oxidation by the liver itself (a relative decrease in carbohydrate oxidation in the liver). This is compensated for by an acceleration in the catabolism of fat and protein (gluconeogenesis). The oxidation of fatty acids coincident with a relative insufficiency of carbohydrate oxidation is incomplete and ketone bodies are formed. From this point of view, the same mechanism is responsible for ketone formation in all conditions associated with a depletion of liver glycogen (fig. 2).

The fact that the development of hyperthyroidism during the course of diabetes mellitus results in an aggravation of the diabetic condition is to be expected from the above considerations. Because of the pancreatic deficiency, the liver cannot retain glycogen and both a relative decrease in carbohydrate utilization by the liver and gluconeogenesis occur. The glucose arising from the latter process is discharged into the blood resulting in a hyperglycemia. With the onset of hyperthyroidism, an increase in the utilization of carbohydrate occurs in all tissues, leading to a more complete exhaustion of liver glycogen. This must result in a further increase in the rate of fat and protein catabolism by the liver, and, consequently, an exaggeration of the condition observed in each disease alone.

SUMMARY AND CONCLUSIONS

1. The utilization of carbohydrate by the extrahepatic tissues of the thyroid-fed rabbit is much greater than that of normal animals.

2. Since the liver is not present to influence the blood sugar, the increased utilization of glucose by the extrahepatic tissues of the eviscerated, hyperthyroid animal is not secondary to any defect in either the glycogenic or glycogenolytic mechanism of the liver.

3. It is suggested that the similarity between the hyperthyroid and diabetic syndromes is due to the accelerated glycogenolysis which is common to both conditions. This acceleration is probably a primary phenomenon in diabetes and a secondary one in the hyperthyroidism.

We are indebted to Miss Dorothea Hamm for technical assistance.

REFERENCES

- (1) COGGESHALL, H. C. AND J. A. GREENE. *This Journal* **105**: 103, 1933.
- (2) CRAMER, W. AND R. A. KRAUSE. *Proc. Roy. Soc., B.* **86**: 550, 1913.
- (3) SANGER, B. J. AND E. HUN. *Arch. Int. Med.* **30**: 397, 1922.
- (4) CRAMER, W. AND R. M'CALL. *Quart. J. Exper. Physiol.* **12**: 97, 1918.
- (5) RICHARDSON, H. B., S. Z. LEVINE AND E. F. DuBOIS. *J. Biol. Chem.* **67**: 737, 1936.
- (6) JOHNSTON, J. A. *Am. J. Dis. Children* **48**: 1015, 1934.
- (7) ANDRUS, E. C. AND D. McEACHERN. *Trans. Assoc. Am. Phys.* **49**: 65, 1934.
- (8) McMASTER, P. D. AND D. R. DRURY. *J. Exper. Med.*, **49**: 745, 1929.
- (9) MARKOWITZ, J. W., W. M. YATER AND W. H. BURROWS. *J. Lab. Clin. Med.* **18**: 127, 1933.
- (10) DRURY, D. R. *J. Exper. Med.* **49**: 759, 1929.
- (11) MAJOR, S. G. AND F. C. MANN. *This Journal* **102**: 409, 1932.
- (12) MIRSKY, I. A. *This Journal* **115**: 424, 1936.
- (13) SOSKIN, S. AND I. A. MIRSKY. *This Journal* **114**: 106, 1935.
- (14) MIRSKY, I. A. *This Journal* **116**: 322, 1936.

A STUDY OF THE BLOOD SUGAR OF THE ADRENAL-ECTOMIZED DOG

W. M. PARKINS,¹ H. W. HAYS AND W. W. SWINGLE

From the Biological Laboratory, Princeton University, Princeton, N. J.

Received for publication May 22, 1936

Britton and Silvette (1932-1935) in a series of studies on rats, cats, marmots, opossums and dogs have vigorously championed the view that the adrenal cortex is a fundamental factor in regulating the metabolism of carbohydrate. According to them, ablation of the cortex leads to rapid depletion of the glycogen stores, the animal loses the ability to mobilize glycogen, the blood glucose falls to extremely low levels and the animal dies in hypoglycemic convulsions. They state, moreover, that injection of adrenal cortical hormone markedly elevates the blood glucose of the intact animal. (The extensive literature on this subject has been adequately reviewed by Wyman and Walker, 1929, and Britton, 1930 and subsequent publications.)

However, not all investigators who have studied the rat and cat agree that hypoglycemia is a constant finding in the adrenalectomized animal or that it plays an important rôle in adrenal insufficiency (Hartman, 1933; Zwemer and Sullivan, 1934).

In recent years those workers who have employed the dog agree that alterations of blood glucose levels in this species following ablation of the adrenals, are of no great significance in so far as the symptom complex resulting from adrenal removal is concerned (Rogoff and Stewart, 1926; Harrop et al., 1933, 1935; Swingle, Piffner, Vars and Parkins, 1934; Kendall, 1935).

The present study is concerned solely with the blood sugar level of the adrenalectomized dog subjected to various experimental procedures. The blood glucose determinations were made on tungstic acid filtrates with the Somogyi modification of the Shaffer-Hartman reagent and the blood samples taken from the femoral artery. In order to conserve space, only the terminal values are given in the tables, although the blood sugar was followed at intervals throughout the insufficiency and recovery periods. The arterial pressures were obtained by use of the needle puncture method (Parkins, 1934).

Blood glucose of adrenalectomized dogs permitted to develop adrenal insuf-

¹ E. R. Squibb and Sons Fellow in the Biological Sciences.

iciency by withholding cortical hormone. A total of twenty-eight cases of uncomplicated insufficiency exhibiting mild to severe symptoms was studied. Since the degree of insufficiency can easily be judged by the level of arterial pressure, these determinations are included in the table.

The data are presented in abbreviated form in table 1. Study of this table reveals that the bilaterally adrenalectomized dog does not usually exhibit marked deviation of the blood glucose from normal. Only a relatively few cases have come under our observation in which really significant lowering of the blood glucose occurs. (Cases 8, 13, 26 and 28,

TABLE 1
The blood glucose of bilaterally adrenalectomized dogs

EXPERIMENT NUMBER	GLUCOSE ON DAY EXTRACT WAS WITHHELD		SEVERE INSUFFICIENCY. CORTICAL HORMONE INJECTED IMMEDIATELY AFTER BLOOD SAM- PLE TAKEN		EXPERIMENT NUMBER	GLUCOSE ON DAY EXTRACT WAS WITHHELD		SEVERE INSUFFICIENCY. CORTICAL HORMONE INJECTED IMMEDIATELY AFTER BLOOD SAM- PLE TAKEN	
	Bp.	Glucose	Bp.	Glucose		Bp.	Glucose	Bp.	Glucose
	mm. Hg	mgm./ 100 cc.	mm. Hg	mgm./ 100 cc.		mm. Hg	mgm./ 100 cc.	mm. Hg	mgm./ 100 cc.
1	100	87	46	83	15	82†	73	68*	66
2	105	76	50	81	16	107	90	56	87
3	98	84	53	85	17	105	80	48	102
4	104	94	50	83	18	98	84	45	69
5	106	82	48	78	19	92	89	50	89
6	103	84	56	80	20	102	80	46	73
7	96	80	36	83	21	108	100	48	89
8	104	77	42	58	22	98	75	60	75
9	100	87	43	89	23	102	78	72	97
10	106	87	70*	87	24	100	80	44	75
11	96	73	60	80	25	98	80	44	80
12	98	75	54	70	26	104	87	53	59
13	83†	71	58	59	27	100	69	55	62
14	104	78	50	73	28	98	69	50	59

* Mild insufficiency.

† Subminimum maintenance dose of extract.

table 1, exhibited the most drastic reductions we have noted.) These are offset by other dogs in insufficiency which showed rises in blood sugar when the animals were in collapse, e.g., cases 2, 11, 17 and 23. Hypoglycemia is an uncommon occurrence in the adrenalectomized dog allowed to develop uncomplicated insufficiency by simply depriving him of cortical hormone. Lowering of the blood sugar levels can, however, be induced in these animals by various experimental procedures which as a general rule do not decrease the blood glucose of the dog with intact adrenals. This fact is illustrated in the following section.

Blood glucose changes following 1, a single stage bilateral adrenalectomy, and 2, muscle trauma to the adrenalectomized dog. Removal of both adrenals at a single stage is a severe operation in the dog and leads to rapid onset of shock and collapse. It has been the experience of workers in this laboratory (Swingle and Parkins, 1934) that dogs so treated rarely survive longer than 24 to 36 hours. Most of the dogs die within the first 24 hours following gland ablation unless treated with cortical hormone or large doses of concentrated sodium chloride and hormone. The serum electrolyte changes and altered fluid distribution occurring in these animals are similar, however, to those observed in uncomplicated adrenal insufficiency following extract withdrawal. The method of operation has been previously described and need not concern us here. Table 2 gives the pertinent data

TABLE 2

Changes in blood glucose following bilateral adrenalectomy at a single stage operation

EXPERIMENT NUMBER	BEFORE OPERATION		SHOCK AND COLLAPSE 10-26 HOURS AFTER OPERATION		REMARKS
	Bp.	Glucose	Bp.	Glucose	
	mm. Hg	mgm./ 100 cc.	mm. Hg	mgm./ 100 cc.	
1	117*	82	42	64	Recovery on hormone treatment
2	108	82	40	84	No treatment given. Death
3	106	84	54	64	No treatment given. Death
4	104	84	43	44	Injected with saline. Death
5	120*	87	56	80	Injected with saline plus extract. Recovery
6	124*	86	45	75	Injected with saline. Death
7	120*	87	45	66	Injected with saline plus extract. Recovery
8	118*	84	44	43	Injected with saline. Death

* Animals untrained for blood pressure work. Remainder of dogs trained.

on the blood glucose changes following removal of both adrenals at a single stage operation where no treatment of any kind was given or else the treatment was begun after the glucose sample had been taken.

In general the blood glucose tends to fall following the single stage bilateral operation, but with the exception of cases 4 and 8 (table 2) significant hypoglycemic levels were not reached even at death. Several of the dogs revealed negligible changes in blood sugar (expts. 2 and 5) when in profound shock and collapse. It would seem that the bilateral adrenalectomy at a single stage, by suddenly depriving the animal of all adrenal tissue, plus the trauma and shock of the double operation, does in some cases upset the balance of endocrine and possibly other non-hormonal factors regulating carbohydrate metabolism and hypoglycemic changes

may result. However, such changes are by no means constant, as shown in table 1. The adrenalectomized dog not receiving hormone treatment rarely presents significant changes in blood sugar even when moribund from insufficiency, when trauma and shock are not complicating factors.

It is of interest in this connection, to note that the tendency toward hypoglycemic changes which the animals sometimes exhibit can be con-

TABLE 3

Effect of adrenalin injections upon the blood glucose of dogs bilaterally adrenalectomized at a single stage operation

EXPERIMENT NUMBER	BEFORE OPERATION		TIME AFTER OPERATION	AFTER OPERATION		SYMPTOM	REMARKS
	Bp.*	Glucose		Bp.	Glucose		
	mm. Hg	mgm./ 100 cc.	hours	mm. Hg	mgm./ 100 cc.		
1	116	66	26	55	93	Shock, collapse	Adrenalin, 4.8 cc. 1:10,000 every 4 hours, subcutaneously. Death
2	118	84	22	40	69	Shock, collapse	Adrenalin, one injection 5.8 cc. 1:10,000. Extract injected. Recovery
3	115	78	16	60	80	Shock symptoms	Adrenalin, one injection 5.8 cc. 1:10,000. Extract injected. Recovery
4	112	84	27	28	73	Collapse	Adrenalin. Large doses every few hours. Death
5	120	84	15	34	48	Collapse	Adrenalin, 24 cc. 1:10,000 given in divided doses over 15-hour period. Death
6	121	84	26	58	71	Marked symptoms	Adrenalin 21 cc. 1:10,000 given in divided doses over 26-hour period. Death
7	110	80	25	36	98	Collapse	Adrenalin. Large doses every few hours. Death
8	116	87	19	45	75	Collapse	Adrenalin, 10 cc. 1:10,000 given in 2 doses over 19-hour interval. Death

* Animals not thoroughly trained for blood pressure determinations.

trolled by injecting adequate amounts of adrenalin. Such treatment, in the absence of cortical hormone, does not prolong the life-span of the animal, nor does it alleviate the shock symptoms, but if given in sufficient doses adrenalin does elevate the blood sugar to levels higher than the normal for the animal or else maintains it within the range of normal. Dogs so treated die in shock with a normal or elevated blood glucose. The essential data regarding this type of experiment are given in table 3. Only

those cases showing a definite tendency toward hypoglycemia were used for adrenalin injections. One animal failed to respond to the injections.

The amount of adrenalin required to bring about changes in blood glucose in this type of experimental animal is greater than that requisite for induction of similar changes in the normal intact animal. This is not peculiar to the adrenalectomized dog for it has been demonstrated by Cope

TABLE 4

Effect of muscle trauma upon the blood glucose of the healthy, vigorous, adrenalectomized dog

EXPERIMENT NUM- BER	TIME	GLU- COSE	SYMPTOMS	Bp.	REMARKS
		<i>mgm./ 100 cc.</i>		<i>mm. Hg</i>	
1	10:20 a.m.	73	Normal	94	Traumatized
	1:10 p.m.	69	Deep shock	42	Death. No treatment given
2	8:45 a.m.	84	Normal	100	Traumatized
	12:15 p.m.	127	Shock		
	2:10 p.m.	213	Deep shock	38	Death. No treatment given
3	9:30 a.m.	71	Normal	98	Traumatized
	4:02 p.m.	73	Deep shock	32	Death within hour after sample taken. No treatment
4	9:50 a.m.	69	Normal	110	Traumatized
	2:45 p.m.	69	Weak	60	
	11:40 p.m.	59	Shock	52	Injection cortical hormone. Recovery
5	10:50 a.m.	82	Normal	110	Normal health and vigor. Traumatized
	7:30 p.m.	64	Normal	84	
	10:00 p.m.	50	Shock	50	Injection cortical hormone. Recovery
6	10:00 a.m.	80	Normal	108	Traumatized
	11:10 a.m.	80	Normal	68	
	3:00 p.m.	50	Shock	47	Injection cortical hormone. Recovery

and Marks (1934), that the effect of adrenalin injections in inducing hyperglycemia is greatly diminished following hypophysectomy. It is evident, however, that the shock resulting from the single stage bilateral adrenalectomy in the dog is not due to hypoglycemia.

In an earlier paper (Swingle and Parkins, 1935) it was observed that the shock syndrome following muscle trauma to healthy, vigorous, adrenalectomized

tomized dogs on maintenance doses of cortical hormone was occasionally complicated by considerable reduction in the level of blood sugar. The data from this type of experiment are recorded in table 4. The blood sugar changes were quite variable. Some animals even at death reveal no significant changes, others show elevation and still others sharp decreases.

TABLE 5

The blood glucose of the shocked adrenalectomized dog treated with adrenalin

EXPERIMENT NUMBER	TIME	GLUCOSE	SYMPTOMS	Bp.	REMARKS
		<i>mgm./100 cc.</i>		<i>mm. Hg</i>	
1	10:20 a.m.	80	None	110	Normal health and vigor. Muscle trauma
	1:00 p.m.	80	None	98	
	3:30 p.m.	111	Marked	56	Injected with 6.4 cc. 1:10,000 adrenalin at 3:00 p.m.
	3:55 p.m.	202	Collapse	36	Died few minutes later
2	9:30 a.m.	84	None	103	Normal health. Traumatized
	2:00 p.m.	91	Slight	84	Injected with 5.8 cc. 1:10,000 adrenalin at 11:00 a.m., 5:00 p.m. and 7:30 p.m.
	7:10 p.m.	115	Mild shock	65	
	9:30 p.m.	89	Deep shock	46	Injection cortical hormone. Recovery
3	9:00 a.m.	89	None	109	Normal health. Traumatized
	4:30 p.m.	105	Mild shock	62	Injected with 5 cc. 1:10,000 adrenalin at 10:30 a.m. and 3:00 p.m.
	8:25 p.m.	96	Deep shock	42	Injection cortical hormone. Recovery
4	9:30 a.m.	82	None	103	Normal health. Traumatized
	1:30 p.m.	75	Moderate	70	
	5:00 p.m.	64	Marked	60	Injected with 5.5 cc. 1:10,000 adrenalin at 5:15 p.m. and again at 8:40 p.m.
	10:10 p.m.	91	Deep shock	48	Injection cortical hormone. Recovery

It proved to be a simple matter to control the level of blood sugar in these traumatized dogs by injecting adequate amounts of adrenalin. The blood glucose was thereby elevated above the normal but this was without effect upon the shock symptoms and it was necessary to inject cortical hormone in order to save the animals. Table 5 gives the essential data.

These experiments afford a probable explanation for the occasional abrupt decline in blood sugar following bilateral adrenalectomy at a single stage. The trauma incident to the double operation is apparently a contributing factor to the fall in blood sugar in these animals.

Effect of intravenous and intraperitoneal injections of large amounts of cortical hormone upon the blood sugar of the adrenalectomized dog. Six animals were studied with the view of determining whether or not injections of large amounts of cortical hormone have any significant effect upon the blood sugar level of the adrenalectomized dog prostrate from severe insufficiency and the healthy, vigorous dog lacking adrenals. Table 6 gives the pertinent data. The changes in blood sugar levels in both types of experimental animal do not appear to be important. Some of the animals may exhibit a few milligrams rise in blood glucose shortly after injection, whereas others do not show such changes. It will be observed that the amounts of hormone administered were considerable. Britton et al. (1932) and Zwemer and Sullivan (1934) maintain that the blood sugar is markedly elevated even in the normal animal when large doses of cortical hormone are injected. Harrop et al. (1933) were unable to confirm these observations. Our data agree with those of Harrop. In drawing conclusions from this type of experiment it should always be borne in mind that cortical extracts contain traces of adrenalin. The adrenalin content of the stock extracts employed in this laboratory varies between 1:500,000 and 1:1,000,000 as determined by blood pressure assay.

Blood glucose changes in the adrenalectomized dog in shock and collapse following intraperitoneal injections of isotonic glucose. The writers have elsewhere presented cogent reasons for assuming that the dog in insufficiency and the animal in collapse following intraperitoneal injections of glucose are physiologically comparable (Swingle, Parkins and Taylor, 1936, in press). If this assumption is valid then the blood sugar of the glucose-injected animals during prostration becomes of great significance. The data relating to this set of experiments are presented in table 7. It is obvious that the intraperitoneal injections of glucose raises the blood sugar but at the same time throws the animal into shock and collapse from dehydration. The recovery of these animals depends, not on the level of blood glucose, but whether or not normal internal osmotic relations are reestablished either by injections of cortical hormone or concentrated sodium chloride.

Blood sugar of the adrenalectomized dog during oestrus (pseudopregnancy) and not receiving cortical hormone. The essential data obtained from study of four representative cases are shown in table 8. The animals employed were brought into oestrus by daily injection of extract of menopause urine²

² We are indebted to Dr. J. A. Morrell of E. R. Squibb and Sons for generous supplies of extract of menopause urine.

(25-50 units daily) for 6 to 10 days. Following the onset of oestrus the bitches were bred to a vasectomized male and the daily injections of cortical hormone and menopause urine extract discontinued. Blood sugar samples were drawn at 7 to 10 day intervals throughout the experiments.

TABLE 6

Effect of intravenous injection of large doses of cortical hormone upon the blood glucose of the adrenalectomized dog

EXPERIMENT NUMBER	TIME	GLU- COSE	SYMPTOMS	Bp.	REMARKS
Adrenal insufficiency					
1	11:45 a.m.	80	Severe insuf- ficiency	44	34 cc. extract (3 cc./kgm.)
	1:50 p.m.	82			
	3:20 p.m.	84			
2	4:10 p.m.	82	Severe	48	28.8 cc. extract (3 cc./kgm.)
	8:00 p.m.	78			
3	12:00 m.	64	Mild insuffi- ciency	66	30 cc. extract (3 cc./kgm.)
	2:00 p.m.	71			
	4:00 p.m.	80	None	88	
4	10:00 a.m.	89	Severe	50	26 cc. extract (3 cc./kgm.) 4/20/35
	12:00 m.	89			
	1:00 p.m.	96	Slight im- provement	57	
	2:00 p.m.	93			
	10:00 a.m.	96	Marked im- provement	76	4/21/35
Normal health					
5	10:20 a.m.	64	None	97	12 cc. extract (1 cc./kgm.)
	12:15 p.m.	73	None	100	
6	10:00 a.m.	84	None	109	30 cc. extract (3 cc./kgm.)
	12:30 p.m.	89			
	2:30 p.m.	87			
	4:30 p.m.	84	None	107	

During the period of normal health and vigor which in our experience may vary from 40 to 60 days, the blood sugar levels tend to be higher than normal. There is some fluctuation near the end of the pseudopregnant period, however, and in one case (table 8) the blood glucose at the termina-

tion of the experiment when the animal was showing symptoms of insufficiency was somewhat below normal.

The explanation of this elevation of the blood sugar levels during oestrus and pseudopregnancy in the dog is not clear but may possibly have some

TABLE 7

Blood glucose changes in the healthy, vigorous, adrenalectomized dog thrown into shock and collapse by intraperitoneal injections of isotonic glucose

EXPERIMENT NUMBER	BLOOD SUGAR BEFORE GLUCOSE INJECTION		SHOCK AND COLLAPSE 1-9 HOURS AFTER GLUCOSE. HORMONE INJECTION IMMEDIATELY AFTER SAMPLE		REMARKS
	Bp.†	Glucose	Bp.	Glucose	
	mm. Hg	mgm./100 cc.	mm. Hg	mgm./100 cc.	
1	94	73	50	100	Extract injection. Recovery
2	98	64	40	170	No extract. Death
3	102	80	44	131	Extract injection. Recovery
4	103	87	50	102	Extract injection. Recovery
5	84*	72	26	96	No extract. Death
6	90	80	50	82	Extract injection. Recovery
7	100	89	62	140	Extract injection. Recovery

* Subminimum maintenance dose of extract.

† The dogs were thoroughly trained for blood pressure work.

TABLE 8

Blood glucose of adrenalectomized dogs during period of oestrus (and pseudopregnancy) and not receiving cortical hormone

DOG NUMBER	DATE	GLUCOSE BEFORE EXTRACT DISCONTINUANCE	DAYS OFF EXTRACT	GLUCOSE, ANIMAL IN INSUFFICIENCY*	Bp.	REMARKS
		mgm./100 cc.		mgm./100 cc.	mm. Hg	
1	2/23/36	82	43	96	56	Severe insufficiency. Extract injected
2	2/11/36	84	58	89	75	Mild insufficiency. Extract injected
3	4/17/35	76	40	60	50	Severe insufficiency. Extract injected
4	3/ 4/36	100	52	87	48	Severe insufficiency. Extract injected

* During the period of normal health the blood sugar was higher than these terminal figures indicate.

relation to the anterior pituitary. The work of Houssay and Biassotti (1931), Barnes and Regan (1933), Long and Lukens (1936) and others definitely shows that the anterior pituitary is intimately concerned with

carbohydrate metabolism. The drastic fall in blood sugar levels following hypophysectomy in the monkey has been recently demonstrated by Smith (1936).

SUMMARY AND CONCLUSIONS

1. The healthy, vigorous, adrenalectomized dog permitted to develop severe insufficiency by withholding cortical hormone does not usually exhibit significant deviations of the blood glucose from normal.

2. The dog bilaterally adrenalectomized at a single stage operation and the traumatized adrenalectomized animal may show sharp reductions in the blood sugar when in collapse. The blood glucose changes in these types of experimental animal are, however, inconstant and extremely variable.

3. Adrenalin, injected into such animals as reveal a tendency for the blood glucose to fall when subjected to the procedures mentioned above, raises the blood sugar to normal or considerably above normal. The high glucose has no effect upon the shock symptoms.

4. Intravenous or intraperitoneal injections of large amounts of cortical hormone of high unitage have no significant effect upon the blood sugar level of either the healthy, vigorous, adrenalectomized dog, or the animal prostrate from insufficiency.

5. Intraperitoneal injections of isotonic glucose into healthy, vigorous, adrenalectomized dogs on a maintenance dose of hormone induces shock and collapse. Animals so treated die (unless injected with cortical hormone or concentrated salt) with blood sugar levels far above the normal.

6. The adrenalectomized bitch in oestrus (pseudopregnancy) maintains herself in normal health for 40 to 60 days without cortical hormone. During this interval the blood glucose is generally higher than normal.

7. The adrenal cortical hormone *per se* is apparently not directly concerned with the metabolism of carbohydrate, at any rate, in so far as this is reflected by changes in the blood glucose levels of the adrenalectomized dog.

8. Hypoglycemia is not a significant factor in adrenal insufficiency in this species.

REFERENCES

- BARNES, B. O. AND G. F. REGAN. *Endocrinol.* **17**: 522, 1933.
BRITTON, S. W. *Physiol. Reviews* **10**: 617, 1930.
BRITTON, S. W. AND H. SILVETTE. *This Journal* **99**: 15, 1931; **100**: 701, 1932; **100**: 693, 1932.
Science **82**: 230, 1935.
COPE, O. AND H. P. MARKS. *J. Physiol.* **83**: 157, 1934.
HARROP, G. A. AND A. WEINSTEIN. *J. Exper. Med.* **57**: 305, 1933.
HARROP, G. A., L. J. SOFFER, W. M. NICHOLSON AND M. STRAUSS. *J. Exper. Med.* **61**: 839, 1935.

- HARTMAN, F. A. *Ann. Int. Med.* **7**: 6, 1933.
- HOUSSAY, B. A. AND A. BIASSOTTI. *Endocrinol.* **15**: 511, 1931.
Pflüger's Arch. **227**: 239, 1931.
- KENDALL, E. C. *J. A. M. A.* **105**: 1486, 1935.
- LONG, C. H. AND F. W. LUKENS. *J. Exper. Med.* **63**: 465, 1936.
- PARKINS, W. M. *This Journal* **107**: 518, 1934.
- ROGOFF, G. M. AND G. N. STEWART. *This Journal* **78**: 711, 1926.
This Journal **86**: 20, 1928.
- SILVETTE, H. *This Journal* **108**: 535, 1934.
- SMITH, P. E., L. DOTTI, H. H. TYNDALE AND E. T. ENGLE. *Proc. Soc. Exper. Biol. and Med.* **34**: 250, 1936.
- SOMOGYI, M. *J. Biol. Chem.* **70**: 599, 1926.
- SWINGLE, W. W., J. J. PFIFFNER, H. M. VARS AND W. M. PARKINS. *This Journal* **107**: 259, 1934; **108**: 144, 1934.
- SWINGLE, W. W. AND W. M. PARKINS. *This Journal* **111**: 426, 1935.
- SWINGLE, W. W., W. M. PARKINS AND A. R. TAYLOR. *This Journal* (in press).
- WYMAN, L. C. AND B. S. WALKER. *This Journal* **89**: 215, 1929.
- ZWEMER, R. L. AND R. C. SULLIVAN. *Endocrinol.* **18**: 730, 1934.

DEPRESSION IN ORDER OF FREQUENCY OF THE ELECTRICAL COCHLEAR RESPONSE OF CATS¹

EDMUND P. FOWLER, JR. AND T. W. FORBES

From the Department of Pathology and Otolaryngology, College of Physicians and Surgeons, Columbia University, and from New York State Psychiatric Institute

Received for publication May 22, 1936

Since the discovery of the electrical response of the ear by Wever and Bray (1930), various experimenters have investigated the mechanism by which it arises in an effort to elucidate the mechanism of hearing. It has been shown that there are two electrical potential effects, one of which is obtained from the auditory nerve and the second from the cochlea, each having its own characteristics (Davis, Derbyshire and Saul, 1933; Davis and Saul, 1933).

Attempts to obtain light on the mechanism of the electrical response have involved the use of drugs (Adrian, Bronk and Phillips, 1934), and exposure to loud tone (Finch and Culler, 1934; Wever, Bray and Horton, 1934; Horton, 1934; Davis, Derbyshire, Kemp and Lurie, 1935). The response has been totally abolished by use of sodium chloride crystals placed on the membrane of the round window (Hallpike and Rawdon-Smith, 1934).

The last named investigators reported the elimination by sodium chloride of all frequencies. In connection with an experiment designed to see whether injection of quinine di-hydrochloride produced any loss in electrical response, the effect of sodium chloride crystals on the round window which was incidentally mentioned by the above authors, was corroborated. Furthermore, it was noted that the elimination of the cochlear response apparently took place earlier in the higher frequencies than in the lower.

In a preliminary paper we have recorded this differential elimination of frequencies (Fowler and Forbes, 1935). The present paper reports the detailed study with medium intensities of this progressive elimination from high frequencies to low by various agents and different concentrations as reflected in the electrical cochlear response of the cat. Corroboratory

¹ The study was made possible by grants from the Research Council of the American Otological Society and the Hayden Coakley Fund, the facilities of the Department of Pathology, of the College of Physicians and Surgeons of Columbia University and of the Department of Psychology of New York State Psychiatric Institute and Hospital.

histological evidence of progressive damage paralleling the electrical loss will be noted briefly and will be presented in detail elsewhere.

The evidence tends to support a "place theory" of hearing.

METHOD. Apparatus. The intensity of the electrical cochlear response was measured by recording the degree of amplification necessary to make the response just audible in the observer's head phones.

Two different capacitance-coupled amplifiers were used at different times. The first gave a maximum of approximately 80,000 times voltage amplification and the second approximately 100,000 times voltage amplification. Both amplifiers were designed to give a fairly flat amplification characteristic (± 2 db.) from 20 to 15,000 cycles. Head phones were used.

The amplifiers were standardized with a test signal controlled by an attenuator calibrated in decibels. For a given amplifier setting, the signal was adjusted to threshold audibility with the identical phones and the

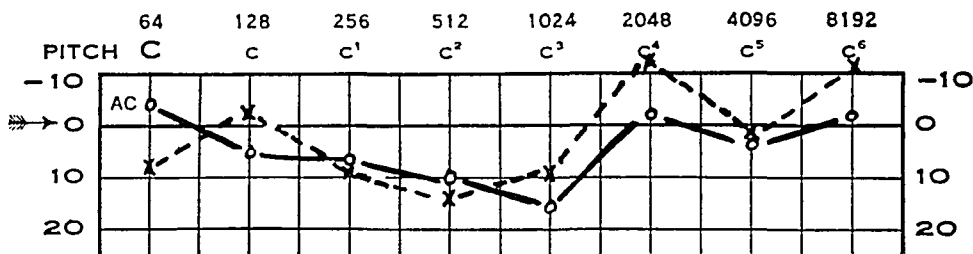


Fig. 1. Observer's audiogram

same observer as the experimental readings. In this fashion any idiosyncrasy of the amplifier-phone combination or of the observer's own audiogram was eliminated. A curve was run for each of the frequencies used. The observer's audiogram is given in figure 1 for completeness.

Electrodes were a zinc plated clip on exposed muscle (grounded) and a copper, sodium chloride active electrode placed on bone at the edge of the round window. This electrode had an insulating handle of glass tubing drawn to a fine tip and a gauze tip about 2 mm. in diameter to preserve moist contact and prevent damage. A shielded concentric electrode was tried but found unnecessary.

Fair sound proofing was obtained by placing the observer three rooms from the operating rooms and closing intervening doors and windows. Observer and operator communicated with each other by visual signals.

The auditory stimuli used were those readily available which were not possible sources of electrical artifact. Tones were obtained from an ordinary harmonica as low as approximately 256 while the higher tones were obtained from a Galton whistle set at readings of 10, 8, 5 and 3 which corresponded roughly to frequencies of 1000, 3000, 5000 and 10,000 re-

spectively. The observer adjusted the amplifier controls until the tone was just audible, and the amplifier settings thus obtained were translated into decibels of amplification from the appropriate standardization curve.

Agents were for the most part applied to the round window membrane either by dropping powder or a drop of the solution upon it. In some of the experiments the entire middle ear was filled with the experimental solution. In either case the ear was syringed out with normal saline and carefully sponged before each set of readings.

Operative exposure. Intraperitoneal pernoston anesthesia (approximately 0.6 cc. of 10 per cent solution per kgm.) was used. The operative exposure of the middle ear was for the most part through a diagonal incision over the lower part of the parotid. With the animal's head on one side the parotid was exposed and its lower end dissected free and retracted. The bulla was then exposed by incision through the muscle directly over it, and a small window was cut in the bulla.

This method seemed superior to the technique which has been often used of exposing the bulla from a mid-line incision, since it occasioned the tying of no vessels. It also had the advantage of permitting a direct view of the round window membrane and the material placed in the niche of the round window remained there to better advantage when the cat's head was on one side.

For sterile operations and for checking the magnitude of electrical leakage from the opposite ear, the bullae were opened by the usual technique from a mid-line incision and two electrodes placed simultaneously in each side.

Histological method. The majority of the animals which were subjected to histological examination were perfused with normal saline followed by 10 per cent formalin, before removal of the temporal bones. After this they were further fixed with 10 per cent formalin. The usual hardening, decalcification and imbedding in celloidin followed. Every tenth section was stained.

RESULTS. Results are reported on a total of 31 cats, of which a preliminary series of 7 animals lacked a normal control ear. It was this series in which the characteristic high tone loss was originally noticed. The results are plotted in terms of the decibels of loss after the application of the experimental agent. The original set of readings after both ears had been exposed but before the agent had been applied, was used as zero loss.

Preliminary series. In the preliminary series intravenous quinine dihydrochloride produced no observable cochlear loss and no electrical response resembling tinnitus was noted. Sodium chloride crystals applied to the round window produced reduction of and elimination of the response, thus confirming Hallpike and Rawdon-Smith (1934). It was

further found that calcium chloride crystals and boric acid also eliminated the response as well as quinine when applied to the window membrane. The elimination was quite rapid, but it seemed that the loss appeared first in the high tones. Since both ears of the animal were used as experimental ears there was no control against possible artifacts from operative trauma, etc., and the results are considered merely exploratory.

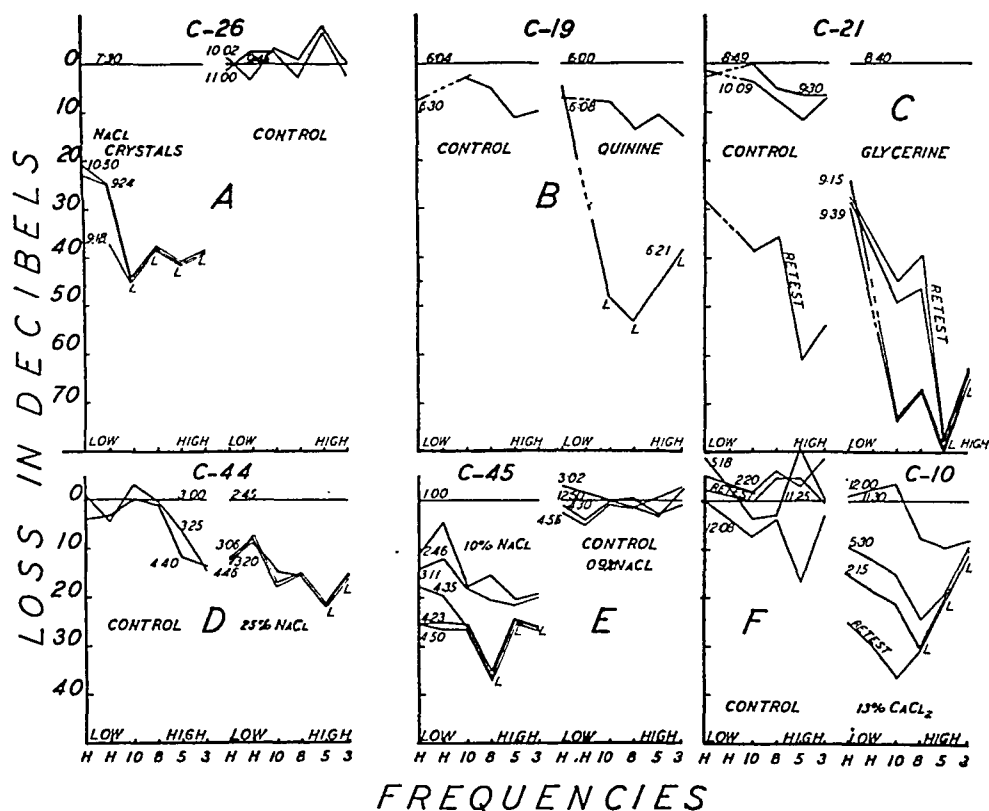


Fig. 2. Illustrating progressive differential loss. Frequencies = 256, 1000, 3000, 5000, and 10,000 respectively (H to setting 3). Left and right ear plotted on corresponding side of each diagram. L = limit of amplification reached. Agent indicated for experimental ear, control ear so marked.

Main series. In the remaining 24 animals the best ear was chosen for experimental purposes and the ear showing the poorer initial electrical response was operated and kept untreated as a control. All agents in the following sections were applied to the round window membrane.

Effective agents. Concentrated sodium chloride, quinine di-hydrochloride, glycerine, and calcium chloride produced a typical picture of cochlear loss which occurred first in the higher tones and later in the lower tones. Figure 2 shows the most typical records of such cases. Figure 2A, B, and C, indicate the effect of the concentrated agents. It will be noted

that in figure 2B and C, the loss is quite evidently progressive as frequency increases, while in figure 2A the increasing extent of loss with higher frequency is masked due to the fact that for the higher tones the limit of amplification is reached sooner than for the lower tones. This was due usually to a poorer initial response to higher tones. Older animals exhibited this to a greater extent than young ones.

With less concentrated agents, i.e., 25 per cent sodium chloride, 10 per cent sodium chloride and 13 per cent calcium chloride, the loss occurred more slowly and the loss in decibels showed a more nearly linear relationship with frequency (fig. 2D, E and F). As shown in figure 2E and F there was also fairly consistent increase of this loss with the time during which the agent was applied to the round window membrane, which loss

TABLE 1
Consistency of characteristic high tone loss

AGENTS	NUMBER OF ANIMALS		OBSERVATIONS ON ABERRANT ANIMALS
	Showing characteristic high tone loss	General loss	
Quinine di-hydrochloride	1	1	Ear full of blood muscular tremor No early reading. High tones poor originally
NaCl crystals	4, 3*†	2.	
NaCl 10 and 25 per cent CaCl ₂ , crystals and 13 per cent Glycerine	3 1, 1*† 2	1 1	High tones poor originally

* From preliminary series.

† Corroborated by preliminary data on dogs.

did not change the general slope of the curve until the limit of recording was reached. Thus these two records allow us to follow the course of the process to better advantage.

Recovery. We obtained some evidence which indicated lack of recovery after from 1 to 25 days. In cat 21 (fig. 2C) there was a very extreme loss with glycerine at 9:39 whereas the control ear was comparatively unaffected as late as 10:09. On retest after 17 days this animal showed approximately the same loss in the experimental ear. Unfortunately at the time of retest the control ear was infected and also showed a gross loss, but in similar records in several other animals the control ear retained its sensitivity and the experimental ear its loss.

The variability of the control ear in figure 2F shows the extent of variation produced at times by none too favorable noise conditions in

the observer's vicinity. In spite of this variability it will be noted that the experimental ear shows two very similar curves at 2:15 and 5:30 and that the control ear shows no loss at 5:18.

Table 1 gives a summary of all the agents producing the characteristic progressive loss with increasing frequency. The table indicates the regular occurrence of the characteristic high tone loss with these agents. Although in 5 of the 24 animals a more general loss apparently occurred, in four of these trauma or other artifact made the record of questionable value.

Ineffective agents. Physiological sodium chloride solution and distilled water showed essentially no effect on the cochlear response. Figure 3A,

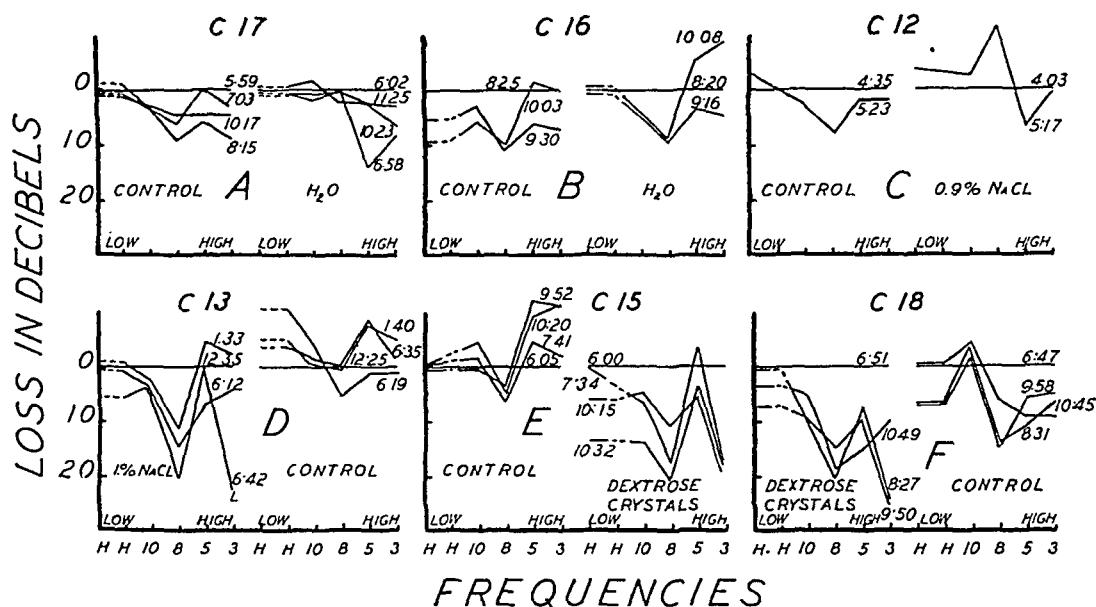


Fig. 3. Illustrating lack of differential loss. Plotting, frequencies and marking as in figure 2. Note that loss is in all cases slight, compared to that shown in figure 2.

B, C, and D, illustrate this fact. It will be noted that in none of the first three is there a loss greater than 10 db. due to either of the above agents and that this is offset by a similar loss in the control ear. The loss shown in figure 3D for one per cent sodium chloride is not consistent nor gross enough in our opinion to be significant for the same reasons.

Dextrose crystals produced a very questionable loss, as shown by figure 3E and 3F. Again the loss was general and not of a gross nature. It is barely possible that there is a slight loss in this case. Table 2 summarizes the results from agents giving essentially negative results.

Histological results. On microscopic examination of six of the experimental animals it was found that there was never any definite bulging of

either the round window or any of the membranes separating the scalae. There was often coagulated debris in the scala media and scala tympani of the experimental ears, but on the whole the main effect of the chemicals seemed to be confined to cells in the scala media. In the acute experiments where the temporal bones were fixed only a few hours after the application of the experimental chemicals, changes were apparent in the hair cells and in the large primitive cells of the external sulcus only. In one animal which was allowed to survive for twenty-five days there was degeneration near the apex of the cochlea in the outer hair cells and in the external cells alone (fig. 4A). Below this the inner hair cell and many more of the sustentacula cells appeared abnormal. Most basalward there was complete destruction of the sustentacula cells, the tunnel of Corti and also of the nerve fibers running in the spiral lamina (fig. 4B). The findings predicate that the electrical potentials of the auditory apparatus are initiated directly or indirectly by the hair cells of the organ of

TABLE 2
Consistency of negative results

AGENTS	NUMBER OF ANIMALS		OBSERVATIONS
	Negative	Inconclusive	
1.0 per cent NaCl	2		
H ₂ O	3		
Dextrose	2	1 (fig. 3F)	Some loss in control

Corti. We are unable to explain the early involvement of the external sulcus but feel that it is interesting in relation to the findings of Crowe, Guild and Polvogt (1934), in human high tone loss cases.

DISCUSSION. *Possible artifacts.* The first and most obvious artifact which would produce a high tone loss of the type which we have found would be an increase in the shunt capacitance due to a change in concentration of the electrolyte either inside or outside of the round window. The latter was eliminated by careful sponging before each series of readings and at any time during the series when the intensity decreased. Furthermore, readings were taken with a 10,000 cycle note immediately following the first low frequency determination as well as in the reverse order so as to obtain the dryest surface during high frequency determinations. Since the reading from the control ears showed lack of high tone loss, such capacitative shunting across the outer surface of the cochlea (Girden, 1934) was evidently avoided.

In order to investigate possible internal electrolytic capacitance changes, a preparation was first tested for cochlear response and then rough meas-



Fig. 4. Illustrating cochlear destruction in a chronic preparation. Cat 28. Agent = NaCl crystals, applied 109 minutes. Animal allowed to survive 25 days, preparation perfused. All sections with 16 mm. objective, 8 X ocular.

A. *Degeneration of hair cells and sustentacula cells from apical turn.* Gray matter in media is hemorrhagic material. Arrows indicate degenerated areas. Dark material above left hand arrow is degenerated material.

B. *Complete degeneration from basal turn.* By comparing with the normal section it will be seen that not only the organ of Corti but also the sustentacula cells and those in the stria vascularis are destroyed.

C = *Normal section from control ear.* Section from basal turn corresponding to B.

urements were made of the two ears with an alternating current bridge at the various frequencies and with the same electrodes. In order to obtain the greatest possible difference between the ears, the loss in one ear was allowed to reach the recordable limit in all but the lowest frequency. As shown in table 3 there was a slight difference of capacitance and resistance between the ears but none great enough to account for the gross loss in the cochlear response.

Artifacts from the frequency characteristics of the amplifier or from the observer's audiogram were controlled by the method of calibration (see p. 25).

TABLE 3

Impedance of ears after gross loss in all frequencies. Cat 24, NaCl crystals

LEFT EAR (EXPERIMENTAL)				RIGHT EAR (CONTROL)		
Frequency	Bridge reading (parallel)		X_{cp}	Bridge reading		X_{cp}
	$C_p(\mu F)$	R_p	ohms	C_p	R_p	ohms
300	0 0674	1300	7875	0.1044	1172	5084
1000	0 0267	785	5964	0.0214	1010	7440
5000	0 0039	650	8165	0.0034	840	9366
10000	0 0019	610	8381	0.0018	735	8846

Decibels loss of cochlear potential response before and after bridge readings

LEFT EAR							RIGHT EAR							
Stimulus frequency								Stimulus frequency						
Time	300	1000	3000	5000	10,000	300		Time	300	1000	3000	5000	10,000	300
8:10	Agent applied													
9:27	41.5	34.5	24.5	9.0+	2.0+	40.0	(Before)	9:31	3.5	2.0	7.0	8.0	0	1.5
11:52	47.0	34.5+	20.0	9.5+	2.0+	33.5	(After)	11:48	1.0	6.0	7.5	4.5	0	-1.5

* The plus sign is used in the table of cochlear loss to indicate the attainment of the limit of amplification. The loss is therefore undoubtedly much greater.

Any effects due to anesthesia or to general traumatic injury, disturbances of circulation, etc., should be reflected in the parallel readings from the opposite untreated ear.

Partial ankylosis of the ossicles by blood clot, or edema of the membrane in the niche of the round window, should cause the opposite effect, i.e., greater loss in low tones.

Type of destruction. In the animals so far studied the experiments indicate that the lesions produced by electrolytes on the round window are similar and perhaps identical with the lesions of so-called senile deafness, drug poisoning and the deafnesses produced by the exposure of the

ear to long continued, very loud tones, since similar pathological findings are present.

Evidence for localization of tones. On the basis of our present evidence and within the limitations of accuracy of our set-up, we believe that the high tone loss is an evidence for localization of the high tones at the basal end of the cochlea and of the lower tones progressively toward the apex. This follows from the fact that the agent perfusing through the window membrane would be more concentrated initially in that region and that the concentration change in the cochlear fluid would be propagated slowly toward the apex. The differential loss thus is indicative of localization similar to that postulated in "place" theories and is in line with reports suggesting such localization with other techniques (Crowe, Guild, and Polvogt, 1934; Hallpike and Rawdon-Smith, 1934; Davis, Lurie and Stevens, 1935; Culler, 1935).

The mechanism of the loss. The loss produced might be supposed to be a matter of osmotic pressure differences, since concentrated solutions and glycerine produced the characteristic loss. However, if such is the case, it is necessary to explain the fact that pure water and dextrose produced practically no loss. Furthermore, there is no evidence from the histological preparations of a maintained pressure difference either in respect to the membranes dividing the scala or in the round window itself.

At present the best tentative hazard at the mechanism producing the loss seems to be that the agents produced a change of permeability of hair cell membranes and that this interfered with the setting up of a chemical concentration difference between the cell interior and scala media, or with release of ions so accumulated. Either a chemical mediator, such as that postulated by Derbyshire and Davis (1935), or cell membrane depolarization effects would be thus interfered with.

It has been shown that an *increase* of permeability of cell membranes of simple forms is produced by supernormal NaCl and CaCl₂ and in the extreme case is accompanied by death of the cell. Differential concentrations of K and Na are necessary to life in these forms. Mechanical stimuli result in permeability changes and exudation of sap from the cell interior (Osterhout, 1922, 1935). It is suggested that an analogous situation in hair cells and sustentacular cells would explain the production in stimulation deafness of destruction like that in our specimens, and reduction of response with the agents used.

Decrease of permeability was obtained by Osterhout with certain agents. We have been unable to show a reversal of sensitivity with CaCl₂ similar to Osterhout's in permeability but this may be due to the roughness of our measure. It is tentatively suggested that the increased permeability effect above noted gives the better explanation of our results.

The source of the cochlear potentials. The occurrence of destruction of

outer hair cells alone with loss in amplitude of cochlear potentials indicates the hair cells to be sources of such potentials.

Apparently both cochlear and nerve processes were actually affected by our agents, since it has been reported that 25 per cent of the amplitude recorded at the round window represents nerve action currents (Derbyshire and Davis, 1935), and it is otherwise impossible to account for so great a loss in our records. If so, the hair cells are apparently direct or indirect initiators also of auditory nerve stimulation. Our results thus support the postulation of numerous investigators. The hair cells are apparently necessary for maximum sensitivity (cochlear potential response) to medium intensities.

A study is now in progress using audiograms of dogs taken by means of a conditioned response technique (Culler, Finch and Girden, 1933), to investigate the effect on hearing.

We are greatly indebted to Mr. Wm. McKnight for aid in preparing the data.

SUMMARY

1. The effect of certain agents on the electrical cochlear response of cats has been investigated by means of the action current technique. The intensity of potentials from the border of the round window, was measured by determining the amplification necessary to make them just audible.

2. Quinine di-hydrochloride, sodium chloride and calcium chloride crystals, sodium chloride and calcium chloride solutions of from 10 to 25 per cent and pure glycerine when applied to the round window produced a characteristic picture in which a gross loss of electrical cochlear response occurred earlier in higher tones than in lower tones. The degree of loss increased with the time that the agents were allowed to remain on the round window. Sections of the cochleae of cats so treated showed various degrees of cochlear degeneration occurring first at the basal end and finally in the apical turn of the cochlea. The extent of the degeneration depended upon the amount of time during which the agents were applied and the time which elapsed between the application of the agents and sacrifice of the animals.

3. Physiological sodium chloride and pure water produced no apparent loss. Similarly dextrose produced a very questionable loss.

4. The characteristic high tone loss is interpreted as evidence for localization along the cochlear spiral of end organs which record tones of medium intensity. Such localization is apparently in order of frequency with the high tones at the basal turn as postulated by Helmholtz, and lower tones progressively toward the apex. Our findings are thus in agreement with those of other investigators using other techniques.

5. In the light of evidence for a chemical mediator from other studies

our results are tentatively interpreted as due to permeability changes which interfere with the formation of or which reduce the liberation of a chemical mediator from the sensory cells. Cell membrane potentials would also be involved.

6. The histologic data indicate that the hair cells are indispensable for maximum sensitivity of cochlear potential response to tones of medium intensity and are primary in the normal activation of the nerve.

REFERENCES

- ADRIAN, E. D., D. W. BRONK AND G. PHILLIPS. *J. Physiol.* **73**: 2, 1931.
DAVIS, H., A. J. DERBYSHIRE, E. H. KEMP AND M. LURIE. *J. Gen. Psychol.* **12**: 251, 1935.
DAVIS, H., A. J. DERBYSHIRE AND L. J. SAUL. *This Journal* **105**: 27, 1933.
DAVIS, H., M. H. LURIE AND S. S. STEVENS. *Ann. Otol., Rhinol. and Laryngol.* **44**: 41, 1935.
DAVIS, H. AND L. J. SAUL. *Am. J. Psychol.* **45**: 358, 1933.
DERBYSHIRE, A. J. AND H. DAVIS. *This Journal* **113**: 476, 1935.
CROWE, S. J., S. R. GUILD AND L. M. POLVOGT. *Bull. Johns Hopkins Hosp.* **54**: 315, 1934.
CULLER, E. *Psychol. Bull.* **32**: 722, 1935.
CULLER, E., G. FINCH, E. GIRDEN AND W. BROGDEN. *J. Gen. Psychol.* **12**: 223, 1935.
FOWLER, E. P., JR. AND T. W. FORBES. *Proc. Soc. Exper. Biol. and Med.* **32**: 827, 1935.
GIRDEN, E. *Psychol. Bull.* **31**: 752, 1935.
HALLPIKE, C. S. AND A. F. RAWDON-SMITH. *J. Physiol.* **81**: 395, 1934.
OSTERHOUT, W. J. V. Injury, recovery and death in relation to conductivity and permeability. *J. B. Lippincott Co., Philadelphia*, 1922.
Collecting Net **10**: no. 1, 1935.
STEVENS, S. S. *Psychol. Bull.* **32**: 723, 1935.
WEVER, E. G. AND C. W. BRAY. *J. Exper. Psychol.* **13**: 373, 1930.

A STUDY OF THE AVERAGE TEMPERATURE OF THE TISSUES, OF THE EXCHANGES OF HEAT AND VASOMOTOR RESPONSES IN MAN BY MEANS OF A BATH CALORIMETER

A. C. BURTON¹ AND H. C. BAZETT

From the Department of Physiology, University of Pennsylvania

Received for publication May 23, 1936

Some of the earliest measurements in animal and human calorimetry were made by observation of the heat given to water baths in which the body was immersed. The great pioneer in the field was Liebermeister (1875) and his pupil Kernig. Considerable improvements in the method were made by Lefèvre (1911) who discussed at length the accuracy and usefulness of the method. Modern human calorimeters make use of an air chamber so that the heat exchanges of the body with its surroundings are those of normal physiology. Since the whole body cannot conveniently be immersed, the usefulness of the water bath as a calorimeter to measure the total heat lost by the body is obviously limited, but for some purposes the water bath possesses advantages over the air chamber, namely, in the study of the mechanism of heat loss by the immersed portion of the body with the surrounding medium.

These advantages arise from the greater efficiency with which heat is exchanged in well stirred water than in air, so that the temperature of the surrounding medium is kept very closely uniform throughout. In addition, the temperature of the surface of the body cannot be more than a fraction of a degree different from that of the water of the bath and may be made to change in any desired manner by changing the temperature of the water. In air the temperature of the surface of the body varies physiologically over a wide range and it is by no means easy to measure the average surface temperature over a large area of the body. It should be remembered, however, that the production of a uniform surface temperature creates a somewhat abnormal physiological condition, which may be associated with abnormality of reflex responses. As it was desired to study how well, or how badly, the changes in deep rectal temperature of the body could serve as an indication of changes in average temperature of the tissues, factors could be more readily controlled if the surface temperature was kept constant while the rectal temperature was changed, and vice versa. Vasomotor reactions to temperature may arise from either

¹ Fellow of the General Education Board, Rockefeller Foundation.

direct effects of the blood temperature upon the heat regulating center or from sensory impulses from the periphery. If the surface temperature may be controlled by the bath temperature, it is possible to study separately the rôle of these two reflex mechanisms. In the interpretation of heat loss from the surface or in the use of surface temperature as an indication of peripheral blood flow, the evaporation of water from the skin is a complicating factor (Burton, 1934a). From the immersed surface no heat is lost by evaporation, even though loss of water through the skin may continue, and the interpretation of thermal changes is correspondingly more direct.

These reasons led to the adaptation of a bath, used previously in studies of circulatory and respiratory responses to temperature (Bazett, 1924) to measure the heat exchanges of the immersed body with the water.

METHOD AND ITS STANDARDIZATION. The bath is an ordinary household bathtub equipped with a stirring device which draws water through a rubber tube from the "head" end and discharges it at the foot of the bath. The temperature of the bath is controlled by a toluol-mercury regulator which runs the length of the bath down one side. This actuates relays which pass or shut off current from the 110 volts A.C. line through three metal immersion heaters (G.E. Hotpoint—500 watts each), mounted near the stirrer at the foot of the bath; in calorimetric periods one of these suffices to keep the bath at constant temperature. The fluctuations of a mercury-in-glass thermometer at the other side of the bath show that the regulator keeps the temperature of the water at this point constant, within two or three hundredths of a degree centigrade. The level of the water in the bath is kept constant by an overflow device set to a definite level. During the experiments, if it is necessary to remove water, it is siphoned out and may be weighed, so that the volume of water displaced by the subject is known.

The deep rectal temperature of the subject is continuously recorded by means of an electrical resistance thermometer and recording bridge (Leeds and Northrup) accurate to about 0.04°F .

The calorimetric device is a simple addition to the bath. The closing of the contacts of the relay that turns on the heaters also passes current through a self-starting electric clock (Telechron), which thus records the total time that the heater has been on. In a parallel circuit is also a similar clock motor which, during the time in which current passes, turns a small pulley on its shaft at the constant rate of one revolution per minute. A thread passes round this pulley and lowers steadily, while the heater is on, a stylus which marks a sloping line on the moving smoked paper of a kymograph. When the regulator turns off the heater, the clock motor stops and the stylus marks a horizontal line on the record. There is thus a record not only of the total time the heater has been on (by the Telechron

clock) but of the moments at which it was turned on and off by the thermal regulator of the bath. Knowledge of the resistance of the heater units and of the average voltage applied to them (this is recorded during the experiments) enables one to calculate the number of kilocalories supplied by the heater in any given period of elapsed time.

The principle of the measurement of the heat given up by the subject is simple. To maintain the temperature of the bath at a constant level, the heater must supply heat at a rate determined by the difference between the temperature of the water and that of the room. The humidity of the room also may affect the heat loss, but this is minimized in the experiments. When the subject is in the bath, heat is given to the bath from the body, and, consequently, the electric heater is turned on less often by the thermal regulator to make the total heat supplied equal to the heat loss of the bath. Comparison between records taken with the subject in the bath, and control experiments with no subject in the bath, after correction has been made for variation in the heat loss of the bath due to any fluctuations of room conditions, gives the heat supplied by the subject.

In order that the contribution of heat by the subject might be a large proportion of the total heat required to balance the heat loss of the bath, the latter was reduced by the enclosure of the bath by a wooden frame containing a layer, about one inch in thickness, of "Rockwool." About two-thirds of the surface of the bath is covered with a permanent layer of paraffin wax, about one inch in thickness, supported by suitably placed struts. When the subject has entered the bath the remaining surface is covered by a removable section of wax, leaving a small opening for the neck of the subject. This opening is finally closed by a collar of rubber, worn by the subject and sealed to the wax by pouring on a little of the melted wax.

The result is that evaporation from the bath is made very small so that the heat loss of the bath is insignificantly altered by the changes in the humidity of the room that might occur between calorimetric and control periods. With the water temperature in the neighborhood of 35°C. and the room at 25°C. the loss of heat by the bath is of the order of 2.5 kilocalories per minute. Since an adult subject produces and eliminates something like 1 kilocalorie per minute, the change to be expected in the heat supplied by the heater is a large percentage of that supplied in the control period. In fact, if the bath temperature is below 30 or 31°C., the heat given up by the subject, unaided by the heater, may be sufficient to keep constant or gradually raise the water temperature. In such cases the calculation of the amount of heat supplied is possible from a knowledge of the thermal capacity of the bath and its rate of rise of temperature. This measurement is, of course, much less accurate than the first method, due to the large thermal capacity and impossibility of measuring very small

changes of temperature of the water; it was only necessary to resort to it in a few cases during the experiments. Incidentally, it is the method that was used by the older investigators.

Heat production was calculated from the oxygen consumption, measured in a Sanborn, using the factor of 4.8 kilocalories per liter of oxygen.

Control experiments. A series of control experiments was made with the bath temperature regulated at temperatures from 30°C. to 38°C. to find how the heat loss of the bath was related to the temperature of the water and that of the room. Since the amount of circulation of the air affects greatly the heat loss, it was decided throughout the experiments to maintain a steady state of circulation by a fan rather than to rely upon the constancy of unstirred room air. A linear relation was found between the rate of heat loss and the "excess temperature" of the water over that of the room (dry bulb temperature). The humidity of the room made no significant difference to this relation. The following procedure was therefore justified. After a series of periods with the subject in the bath were completed (lasting usually a total of four hours), the bath was resealed with wax in as close an imitation to the original seal as possible and two control periods were run, usually each of one hour duration, at two bath temperatures such that the corresponding excess temperatures included between them any excess temperatures occurring in the experiment. The heat loss in any periods could then be calculated by interpolation. An example will make the procedure clearer.

1. Period with subject in the bath.

<i>Actual elapsed times</i>	<i>Reading of heater clock</i>
A.M. 11:56 :00	22':12''
11:34 :20	18':28''
Subtracting 21':40''	3':44''

The heater was "on," on the average, $\frac{224}{21.67} = 10.33$ seconds per minute of elapsed time. Average voltage on heater = 112.8. Bath temperature 33.57°C. Average room temperature 24.03. Excess temperature = 9.54°C. Then Cals./Min. supplied by heater = $(112.8)^2 \times 0.009105 \times 10.33 = 1.20$ Cals./Min. (0.009105 is a factor involving the resistance of the heater.

2. Control periods. Similarly it was found, after the subject had left the bath
Heater supplied 2.781 Cals./Min. with excess temperature
12.04°C.

Heater supplied 1.501 Cals./Min. with excess temperature
8.02°C.

Correction Factor $\frac{1.28 \text{ Cals./Min. for } 4.02^\circ\text{C.}}{1.28 \text{ Cals./Min. for } 4.02^\circ\text{C.}} = 0.318 \text{ Cals./Min./}^\circ\text{C.}$

In the experimental period (1) above, the excess temperature was 9.54°C. The heat loss in that period was therefore:

$$1.501 + (9.54 - 8.02) \times 0.318 = 1.99 \text{ Cals./Min.}$$

Of this the heater supplied..... 1.20 Cals./Min.

Therefore the subject supplied..... 0.79 Cals./Min.

Thermal capacity of the bath. This was found experimentally by measurement of the number of calories supplied by the heaters in raising the bath from constancy at one temperature to final constancy at a higher temperature, making allowance for the heat lost to the room in the period of transition. The result was 284 ± 4 kilocalories

per degree C. for the effective thermal capacity. As the bath contains 280 liters of water when filled to the constant level, a result of this magnitude was to be expected. For periods with the subject in the bath where the thermal capacity had to be used, a number of calories equal to the liters of water displaced by the subject was subtracted.

Accuracy and thermal lag of the heat measurement. To test the accuracy of the measurements under the conditions in which they were to be made, heat checks were made with a 100 Watt lamp (producing 1.23 Cals./Min.) immersed and sealed into the bath in place of the subject. The changes that occurred in the type of record when the lamp was turned on are illustrated by figure 1. The heater, which has been "off" for, on the average, 1.7 minute and "on" 0.8 minute alternately, changed very rapidly when the lamp was turned on to a routine of "off" about 5.5 minutes and "on" for 0.6 minute. These changes show the decreased rate of cooling of the bath and its increased rate of rise of temperature when the lamp was "on." Five minutes were adequate to cover the period of thermal lag. The simultaneous values of the current through the lamp and the voltage across it were measured, so that the number of calories produced per minute was known. The heat received by the water was calculated from the records exactly as with the subject in the bath. Using twenty minute periods the two quantities agreed within 4 per cent. This accuracy is con-

sidered very satisfactory for so simple a calorimetric device using periods of such short duration.

Surface temperature. It is fundamental to the interpretation of the results that the temperature of the immersed surface of the body should differ from the temperature of the water only by a negligibly small quantity. The great ease with which an immersed body exchanges heat with well stirred water suggests that this is true, but the point was experimentally proved. As the superficial tissues are relatively poor conductors of heat, it is difficult to be sure that a thermocouple passing through the water to the skin might not produce a

local cooling of the point of the body surface with which it was in contact, and so give an erroneous idea of the surface temperature. Instead of the subject, therefore, a hollow cylindrical copper vessel, of length 51 cm. and diameter 16 cm., was immersed in the position of the trunk of the subject in the bath. The great thermal conductivity of copper prevented any possible local cooling by the thermocouple wires. An electric lamp inside the cylinder carried sufficient current to generate heat which was eliminated from the cylinder's surface to the water at a rate equal to 36 calories per square meter/hour. A differential thermocouple recorded the difference of temperature between the surface of the cylinder and the point where the bath thermometer was placed.

It was found that while in unstirred water there was established a temperature difference between these two points greater than $1^{\circ}\text{C}.$, yet, when the stirrer was operating, the temperature difference was less than $0.08^{\circ}\text{C}.$ (the smallest measurable temperature difference). The temperature of the immersed surface of the body may then be taken, without appreciable error, to be equal to that of the bath water in the experiments.

EXPERIMENTAL MATERIAL. The series of experiments here reported consist of 10 bath experiments upon one male subject (HCB), using nine-

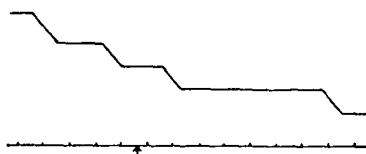


Fig. 1. Record of the time the heater was on and off in a control experiment in which an immersed lamp was turned on at point marked with an arrow. Abscissae: time in minutes. A horizontal line indicates that the heat was off.

teen different bath temperatures and comprising in all 85 periods for which the heat exchange was calculated. One experiment was made with each of two other male subjects (H and G), three experiments on a female subject (M) and one on a second female subject (R). It is obviously not expedient to give the details of this number of experiments. The routine and details of two of the experiments which may be taken as typical are, therefore, given, followed by a brief statement of other experiments. The results obtained are stated in a more general form in the discussion.

Experiment 1. The results of this experiment are shown in figure 2. The subject, in basal condition, entered the bath at 9:40 a.m. The volume of water displaced was measured (60.1 liters), the rectal thermometer adjusted by the subject, and sealing

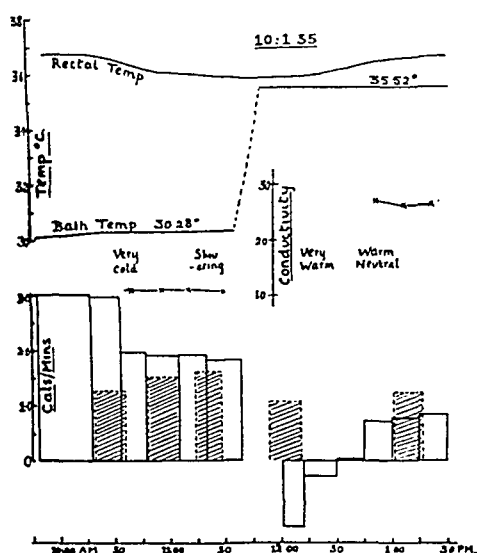


Fig. 2

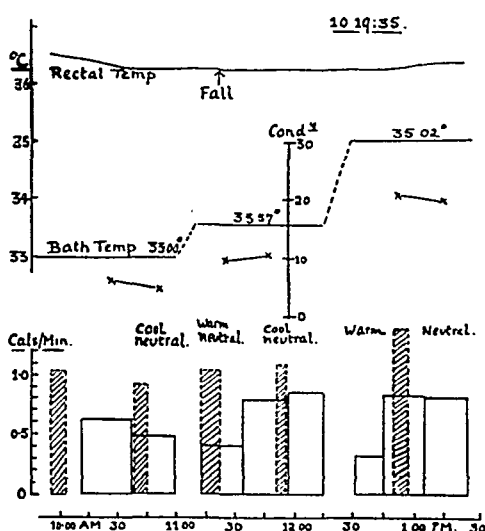


Fig. 3

Fig. 2. Experiment 1. Abscissae: time in minutes; ordinates: temperature of bath and rectum; index of conductivity and calories per minute. The heat given to the bath is indicated by open blocks; that produced, by shaded blocks.

Fig. 3. Experiment 2. Indications as in figure 1.

by wax completed. To reduce the heat loss from the head a rubber bathing cap was worn with a rubber "veil" over the eyes and nose. A preliminary period of ten minutes was necessary to attain steady conditions. In the next thirty minutes the bath temperature rose without any heat being supplied by the heater, as the result of the heat given up by the body in the cooling of the peripheral tissues. The bath regulator was re-adjusted at 30.28°. From this point the bath temperature remained constant; the regulator turned the heater on with increasing frequency as the heat from the body decreased. The rates at which heat was given to the water by the body are shown by the blocks in the figure (full lines), and also in table 1. After the first two periods, a steady state was reached after which the heat given to the bath decreased very little, and this decrease was parallel to the decrease in the difference of temperature between rectal temperature and bath temperature (ΔT in the table). Even without the heat loss from the head, the heat given up by the body to the bath

exceeded the heat production—shown in the diagram by the shaded blocks. The metabolism increased throughout this part of the experiment, while the subject felt cold and eventually shivered.

At 11:40 a.m., the bath temperature was raised in the space of 11 minutes to 35.52°C. by syphoning out some of the cold water and replacing it by hot water from the tap, the level being maintained constant throughout.

As table 1 and the figures show, for the first thirty minutes the heat given up by the subject to the bath was negative, that is, not only was all the metabolic heat conserved by the body, but heat was accepted from the bath water. As will be amplified later in the paper, this could only be the case if the temperature gradients in the peripheral tissues were reversed. The heat exchange finally reached a positive comparatively steady level. Again in the steady state, the heat given up closely paralleled the difference of temperature between rectum and bath. The last column of table 1 is obtained by dividing the heat given to the bath by the subject, expressed

TABLE 1

10:2:35. Subject, HCB. Weight, 67.3 kgm. Entered bath 9:40 a.m.

TIME	RECTAL TEMPERATURE	BATH TEMPERATURE	HEAT TO BATH	HEAT PRODUCED	ΔT	$\frac{H}{\Delta T}$
a.m.			cals./min.	cals./min.		cals./sq.m./hr./°C.
9:50 to 10:18	36.66 to 36.63	30.06	+3.02		6.59	
10:18 to 10:32	36.63 to 36.43	30.22	+2.99	1.29	6.31	
10:32 to 10:47	36.43 to 36.17	30.27	+1.99		6.03	11.0
10:47 to 11:05	36.17 to 36.11	30.28	+1.92	1.53	5.86	11.0
11:05 to 11:20	36.11 to 36.00	30.28	+1.93		5.78	11.0
11:20 to 11:39	36.00 to 35.93	30.29	+1.85	1.63	5.69	10.8
p.m.						
12:01 to 12:12	35.89 to 35.93	35.52	-1.20	1.08	0.39	
12:12 to 12:30	35.93 to 36.14	35.52	-0.18		0.53	
12:30 to 12:45	36.14 to 36.33	35.52	+0.04		0.72	
12:45 to 1:00	36.33 to 36.47	35.52	+0.71		0.88	27.0
1:00 to 1:15	36.47 to 36.56	35.52	+0.79	1.21	1.01	26.0
1:15 to 1:30	36.56 to 36.65	35.52	+0.87		1.09	26.7

in calories per hour per square meter of body surface immersed, by the difference ΔT . Its constancy in the steady state shows that these two quantities are proportional. It will be seen, however, that the value of this constant is greatly increased in the hot bath over that in the cold bath, since the heat has decreased much less than the temperature gradient across which it is flowing. As will be shown later, this indicates an increase in the effective thermal conductivity of the peripheral tissues. The rectal temperature rose, after a lag period, to a comparatively steady temperature.

This experiment illustrates those in the series in which the changes in the bath temperature, and therefore surface temperature, were, physiologically speaking, violent, from a cold bath, where "chemical regulation" by increased metabolism is called upon, to a very warm one where physical regulation by increased peripheral blood flow is inadequate to keep the body temperature from increasing steadily. Presumably, its level would

ultimately rise high enough above the bath temperature to achieve equilibrium. Characteristics to be seen are the large amounts of heat involved in the cooling and heating of the peripheral tissues, the delayed responses of the rectal temperature, and the period of reversed peripheral gradients.

Experiment 2. This is illustrated by figure 2. Here the negative heat exchange indicating reversed peripheral gradients is not evident, though it might occur for a brief period and yet not appear in the calorimetry taken over a longer period. The changes in the effective thermal conductivity are such that when the steady state is reached at the new bath temperature, the heat given up is at approximately the original level. In the case of the first rise of bath temperature, the heat given up to the warmer bath was finally greater than that originally exchanged in the colder bath. Here then the vascular response has over-compensated for the decrease in

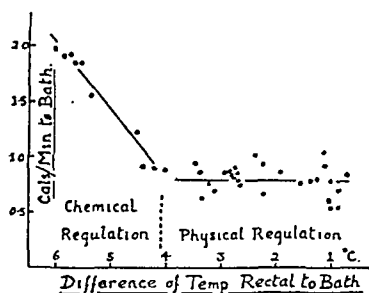


Fig. 4

Fig. 4. Heat given to bath relative to ΔT in subject HCB.

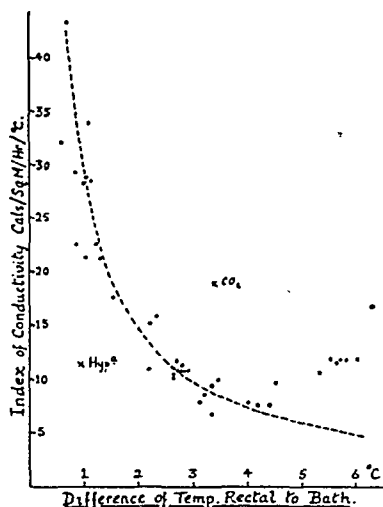


Fig. 5

Fig. 5. Index of conductivity relative to ΔT for subject HCB (summer).

The curve drawn is a theoretical hyperbola, from which the data diverge at high temperature differences.

the difference of temperature (ΔT) between rectal and bath temperatures. In the case of the second rise of bath temperature, the adjustment is quite accurate, the final level of heat exchanged being very close to the original.

In this experiment, which is typical of those where the changes in bath temperature were more moderate, a new phenomenon appears—namely, that an increase in the bath temperature produces an initial fall of rectal temperature, although the calorimetry shows that the body has a positive heat balance and, therefore, that the true average body temperature is rising.

Other subjects gave similar results but showed some individual differences, part of which may have been due to seasonal changes, since, as will

be seen later, even the single subject showed some difference at different times of the year. The area not immersed in the water is a source of variation, and forms a larger proportion of the total surface in the smaller subjects; it amounted, for instance, to 7.8 per cent of a total of 1.8 sq. M. in HCB, while in the smallest subject, M, it was 8.9 per cent of a total of 1.35 sq. M.

RESULTS AND DISCUSSION. *Limits of physical regulation.* The data obtained in the summer months on subject HCB are plotted in figure 4. The ordinates are the heat given to the bath by the subject when the steady state was reached in each instance. With deep to surface temperature differences of 4°C. or less the heat exchange is constant. Over this range the variation to be expected from the changes in the temperature gradients between warmer and colder baths is compensated by physiological adjustments in the effective thermal conductivity of the tissues. With greater temperature differences the compensation is incomplete, heat loss is increased and increased heat production may or may not suffice to prevent lowering of the body temperature.

Effective thermal conductivity index as a measure of peripheral circulation. The constancy of the temperature of a given volume of deep tissues requires a balance between the heat production in that volume and the heat leaving it across its boundaries. The latter follows the fundamental law of heat flow, namely:

$$\text{Flow of heat} = \text{Effective Thermal Conductivity} \times \text{Cross-sectional area} \times \text{Temperature Gradient}$$

(The term "effective thermal conductivity" is not to be thought of as implying "conductivity" in the strict technical sense, but as an index of the ease with which heat flows through tissues by the combined means of direct conduction and "convective" transport of heat by blood flow.) Reflex control of the peripheral blood flow can so alter this index in response to changes of bath temperature, and consequently of gradients, that over the range shown in figure 4 the flow may be kept constant. The vascular response may then be quantitatively expressed in terms of thermal conductivity by transposition of the fundamental equation to the form:

$$\text{Effective Thermal Conductivity} = \text{Heat flow per unit area} / \text{Temperature Gradient}$$

The heat flowing through unit area of the immersed surface to the water is measured in the experiments. The appropriate gradient, if an average value for the thermal conductivity is required, would be the average gradient in the tissues. It can be shown, quite generally, that if two steady states be compared, where the gradients have the same relative distribution with depth in the body, this average gradient is proportional to the difference of temperature between any two chosen depths. Thus a number pro-

portional to the effective thermal conductivity is obtained by dividing the heat given to the bath per unit area by the rectal-surface temperature difference (ΔT of table 1). To obtain the value in the usual units of thermal conductivity this ratio would have to be multiplied by the effective thickness of the tissues through which the heat had flowed.

The values of the effective thermal conductivity for all the summer experiments on subject HCB are shown in figure 5. Only data appropriate to steady states rather than to periods of transition in which gradients were abnormal are used. The dotted curve of figure 5 is a theoretical hyperbola, such that the heat loss (conductivity \times gradient) to the bath would remain constant at 30 kilocalories/sq. M./Hr. Over the range of

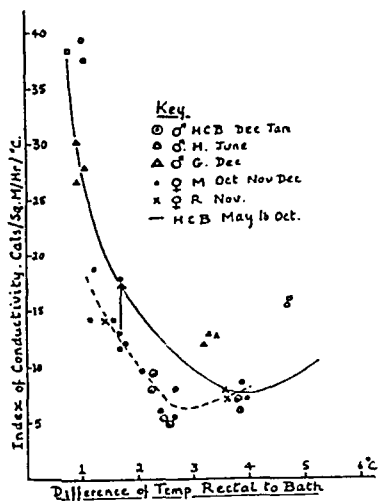


Fig. 6

Fig. 6. Comparison of conductivity indices for other subjects and for winter data on HCB with summer curve for HCB.

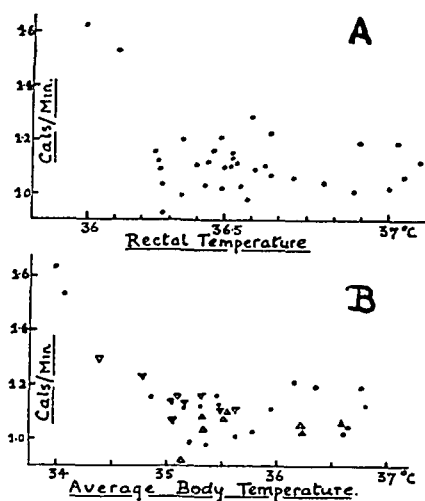


Fig. 7

Fig. 7. A. Relation of heat production to rectal temperature in subject HCB.

B. Relation of heat production to average body temperature in subject HCB.

bath temperatures from 1° to 4° below rectal temperature the points lie close to this line.

In colder baths the effective conductivity after reaching a minimum begins to rise. The minimum coincides with the point at which metabolism begins to increase, eventually leading to shivering. The explanation may then be found in the increased circulation that must accompany increased activity of the peripheral muscles, which would inevitably entail an increase in effective conductivity. Since the blood vessels to the skin pass through the muscles, when cutaneous vasoconstriction is maximal any heating of the blood in its course through muscle must lead to increased heat loss. Thus the method of maintaining body temperature against cold by increased heat production is an inefficient one. Not only has

more energy to be consumed, but in its consumption heat loss is made greater than it would otherwise be. The indices for other subjects and some obtained on subject HCB in the winter are shown in figure 6 for comparison with the summer data on HCB (shown by the full line curve). The data obtained in the winter on subject M, R, and HCB appear to follow a lower curve with a different minimum (shown in the dotted line). On the other hand, one male subject in winter gave data falling on HCB's summer curve. Individual differences undoubtedly exist, but there is evidence also of a seasonal variation. The values for HCB in winter lie significantly below the minimum values obtained in summer. A seasonal variation might be anticipated in view of the known effect of environmental temperature on blood volume, for Barcroft et al. (1922) found increases of 700 cc. to over a litre as a result of exposures to temperatures not significantly different from some common in Philadelphia in the summer. Full vascular dilatation or constriction in the skin as a whole may be difficult to attain without alteration in blood volume, and the slow development of full adjustment to changes in environmental temperature that is common experience may depend in part on an alteration in blood volume.

It was thought that the temperature of the room, which varied between 21° and 27°C., might play a part in determining the vasomotor response to the temperature of the bath, but variations in the average temperature of the face did not produce significant changes in conductivity. With subject M., periods in a bath at constant temperature were compared in which first an electric radiation heater and second a fan were directed on the face. Variations in the average temperature of the face, measured by a light thermocouple, were from a normal of 32.5° to 37.4° and 30.3°. The only significant change of conductivity was produced by the heater, and this effect is shown in figure 6 by the arrow. Variations in room temperature must have acted as stimuli of much lower order.

Figure 5 shows that the effective thermal conductivity of the tissues of HCB was changed by vascular response by a factor of about six times. A lower limit of the conductivity below which that of the tissues could not conceivably fall would be that found for tissues in which there was no blood flow at all. Thermal conductivities of tissues removed from the body were compared with those of an equal thickness of air by Bordier (1898). His results would imply an average figure of about 0.0002 unit (cals./sec./sq. cm./unit temperature gradient), while assuming an "effective thickness" of the tissues of reasonable magnitude, the minimum value obtained in these experiments was of the order of 0.001. An upper limit to the conductivity obviously exists, determined by the maximum blood flow that may be diverted to the periphery of the body. Suppose that to keep in thermal equilibrium 40 calories per sq. M. of body surface per hour must be carried to the surface. In a bath that was one degree cooler than the internal blood temperature, the blood in passing from the heart to the

surface could cool by not more than this one degree. To transfer the heat, a circulation of 40 liters/sq. M./Hour would be the minimum requirement, which is one-third of the total basal cardiac output. The limit to the proportion of the total output that can be diverted to the periphery means that thermal equilibrium cannot readily be attained in a bath less than one degree or so lower in temperature than the body.

Reflex origin of the response. The immediate response to temperature would appear to be mainly reflex in origin, in view of the absence of a relation to changes in rectal temperature. For instance, an increased conductivity accompanied decreased rectal temperature when a cold was followed by a warm bath. Sensations usually showed a similar lack of correlation with the level of rectal temperature and its direction of change. The deep body temperature level or its direction of change can, however, play a part. This was demonstrated in an experiment in which a subject exposed to a bath of 33.7° for over an hour had apparently achieved thermal equilibrium and moderate comfort. At this time a dose of one drop of 1 per cent nitro-glycerine in alcohol was given under the tongue. There resulted a sharp fall of rectal temperature ($0.2^{\circ}\text{C}.$) within two minutes and this was accompanied by temporary intense shivering and increased oxygen consumption. The heat exchange to the bath was increased, though this was not seen until later. Shivering ceased while the rectal temperature remained at its lowest level ($37.2^{\circ}\text{C}.$) presumably because it had ceased to fall or because of some modification of thermal gradients in the region of the receptors. Changes of reflex origin, however, were more prevalent in the experiments, and it may be pointed out that since heat flow is the product of conductivity and thermal gradient, it could theoretically be best controlled if thermal conductivity were regulated by the value of the gradient.

Sensitivity of the method for measuring other circulatory changes. The effect of a small dose of nitroglycerine has been mentioned. Doses of this order were used twice and in each case gave an increase in the thermal conductivity index of about 20 per cent, which reached its maximum in 15 minutes. The pulse rate changes reached their maximum in 4 minutes and had subsided in 8 minutes. The delay in the thermal exchange is attributable to the lag in the temperature changes of the peripheral tissues. Changes in the CO_2 tension of the blood may also produce considerable effects. In one experiment with a bath temperature of 36.3° the subject went into voluntary hyperpnea for 2 minutes; in another, with the bath at 33.0° , he breathed a 10 per cent CO_2 , 90 per cent O_2 mixture for 3 minutes. The results are shown in figure 5 and table 2. With hyperpnea there resulted a sharp rise, with CO_2 a marked fall of rectal temperature, though the degree of muscular respiratory activity was similar; presumably the rectal temperature changes resulted from vasoconstriction and vasodilatation in the skin.

The work of Liljestrand and Magnus (1922) indicated that marked changes in skin circulation might result from baths containing CO_2 and the question arose whether the retention of CO_2 by the paraffin wax cover might modify the skin circulation.

Analysis of the water by a Van Slyke apparatus showed the CO₂ content of the water to be increased to a barely measurable extent, even after the subject had been in the water for several hours. On the other hand, increase in the CO₂ of the water to about 180 mm. produced by bubbling CO₂ through it, or complete removal of CO₂ by the addition of an excess of baryta water produced barely measurable changes (less than 8 per cent) in conductivity and only fleeting sensations of temperature. Accumulation of CO₂ in the water was, therefore, inadequate to produce measurable changes.

Average body temperature. Though the effective thermal conductivity cannot be estimated except in a state that is approximately steady, the initial thermal exchanges between the body and the bath may be measured, and the change in average body temperature be determined. The main sources of error depend on the specific heat capacity assigned to the body, and to the fact that the initial exchanges in the first few minutes, before the bath temperature itself has become steady, cannot be measured. The measurements of heat exchange demonstrate that the superficial tissues

TABLE 2

PERIOD	DURATION OF CALORI- METRIC PERIOD	HEAT GIVEN TO BATH	INDEX OF CONDUCT- TIVITY	AVERAGE INCREASE
	minutes	cals./min'ute		per cent
Control.....	15	0.54	22.4	
Hyperpnea.....	13	0.31	11.9	-46
Control.....	15	0.62	21.3	
Control.....	21	0.73	7.6	
10 per cent CO ₂	18	1.42	15.2	+100

have considerable changes in temperature extending to considerable depths, so that the heat loss or acceptance is large. Though the heat loss from the lungs and head is not measured it may be estimated from the discrepancy between heat production and heat loss in the steady state. If this heat loss from the head, which is relatively small (e.g., 0.25 kilocalorie/min. in subject HCB), be considered constant, the total heat absorbed by the body between steady states at two different bath temperatures may be calculated (e.g., between 11:40 and 12:45 in fig. 2) and the change in average temperature be calculated, since: (heat absorbed) = (weight \times (specific heat capacity) \times (rise of average body temp.). Such estimates may be compared with the heat exchange calculated on the assumption that the change in rectal temperature indicates the change in average body temperature, the usual procedure in calorimetry. The results are shown in table 3. Previous work with Prof. J. R. Murlin by one of us (Burton 1934b) has shown that better agreement is obtained between direct and indirect calorimetry, when a formula, which combines rectal and surface

temperatures, is used to estimate average body temperature. This formula is:

$$(\text{Average body temp.}) = 0.65 (\text{Rectal temp.}) + 0.35 (\text{Surface temp.})$$

The heat so calculated is also shown in table 3.

When the surface changes by large amounts, more than 3°C., the rectal temperature is quite inadequate for the calculation of the heat absorbed,

TABLE 3
Changes in average temperature

OBSERVED CHANGES			HEAT ABSORBED OR LOST				
Duration of period	Rise of rectal temperature	Rise of surface temperature	Measured	Calculated, rectal	Error	Calculated Formula	Error
Major changes							
73	-0.32	-4.62	-119	-17	102	-100	19
101	+0.08	+4.66	+108	+4	104	+91	17
65	-0.10	-3.86	-85	-5	80	-77	8
103	+0.13	+3.87	+113	+7	106	+78	35
66	+0.40	+5.24	+99	+22	77	+113	14
65	+0.77	+4.00	+144	+42	102	+103	41
Totals.....			668		571		134
Minor changes in surface temperature							
43	-0.04	-0.72	-2	-2	0	-11	9
64	-0.03	-0.61	+1	-2	3	-13	14
57	-0.01	+0.56	+15	-1	16	+10	5
50	+0.12	+1.45	+17	+7	10	+32	15
85	-0.04	+1.15	+65	-2	67	+21	44
63	+0.02	+1.17	+61	+1	60	+24	17
198	-0.39	0	+12	-21	33	-14	26
143	-0.36	0	-25	-19	6	-13	12
Totals.....			198		195		142

while the formula gives much better agreement. No adjustment in the value for heat loss from the head could remove the discrepancies. When the change in surface temperature is less than 1.5°, the discrepancies by both methods are less serious, and there is less gain from the use of the formula.

Metabolism and average body temperature. Figure 7 shows the relations between the heat production, as measured by oxygen consumption, and the rectal temperature, and with the average body temperature calculated

by the formula respectively. There is somewhat better correlation with the latter. The scatter may be to some extent dependent on an incomplete attainment of the steady state, so that the formula was not strictly applicable. Consequently, when the bath temperature had been raised less than 30 minutes earlier, the points are indicated by triangles with the vertices uppermost, and vice versa. The distribution of these points suggests that in completely steady states, the scatter would be less. In addition to "chemical regulation" at low temperatures, a rise occurs with increasing average temperature (cf. data of Houghten et al., 1929), though it would be futile to calculate Q_{10} values. The minimum metabolism was

TABLE 4
Changes in rectal temperature following changes in bath temperature

BATH TEMPERATURES	RISE	INITIAL RISE IN RECTAL TEMPERATURE	CHANGE IN SAME DIRECTION—DELAY
			<i>minutes</i>
30.28 to 35.52	+5.24	0	10
32.26 to 36.26	+4.00	0	30
32.12 to 36.01	+3.89	—	16
33.83 to 35.00	+2.17	—	35
33.35 to 35.07	+2.28	—	48
35.07 to 37.01	+1.94	+	0
32.58 to 33.83	+1.25	—	25
33.58 to 35.10	+1.52	—	60
33.57 to 35.02	+1.45	0	28
33.00 to 33.57	+0.57	—	25
35.88 to 31.26	—4.62	+	35
36.00 to 32.12	—3.88	+	15
35.88 to 33.69	—2.19	+	55
35.87 to 34.06	—1.71	0	8
34.34 to 33.62	—0.72	+	45
35.92 to 35.11	—0.81	0	No fall
33.62 to 33.01	—0.61	+	50
35.11 to 34.76	—0.35	+	No fall

Delay measured from the start of change of bath temperature.

found at those bath temperatures which gave the minimum average temperature compatible with an absence of sensations of cold.

Paradoxical changes in rectal temperature. Paradoxical changes in rectal temperature, such as those seen in the exemplary protocols were the rule rather than the exception, so that the rectal temperature might not indicate correctly even the direction of change in average body temperature. When an actual fall of rectal temperature was not seen during warming, the rise was much delayed. A rise of rectal temperature on lowering the bath temperature was also common. The changes are listed in table 4, which also indicates (column 4) the delay between the start of the change in bath temperature and that of a rectal temperature change in

the same direction. This paradoxical change was described by Liebermeister and by Lefèvre (loc. cit.) who considered the possibilities of modified heat distribution but emphasized the metabolic factor on exposure to cold. Since the reflex change in conductivity is likely to take place rapidly, and owing to the thermal capacity of the tissues the new gradient will be established slowly, the product of the two may be expected to change initially in the opposite direction to the change in bath temperature. The paradoxical changes in both directions are then explicable in terms of changed conductivity through vasomotor adjustments, without necessarily any change in heat production. This may be demonstrated mathematically by consideration of a hypothetical physical model.

Physical model—mathematical considerations. In order to study the thermal kinetics of these changes, calculations have been made for a physical model used by one of us (Burton, 1934a) in explaining the thermal gradients of the body in the steady state. This model consists of a cylinder of material of uniform thermal conductivity in which heat is uniformly generated. Solution of the general differential equation for the flow of heat shows that in the steady state the distribution of temperature in the cylinder will be parabolic, being described by the equation

$$\theta_r = \theta_i - \frac{hr^2}{4K}$$

where θ_r is the temperature at radius r from the axis

θ_i is the temperature at the axis

h is the heat generated per unit volume, and

K is the thermal conductivity.

Such a distribution of temperature approximates that found by Bazett and McGlone (1927).

We must substitute the values for the rectal temperature (θ_i) and the surface temperature (θ_s) in one of the bath experiments to find the value of K , the effective thermal conductivity to make the model fit the experiment, i.e., $\theta_i = 36.5^\circ\text{C.}$, $\theta_s = 32.5^\circ\text{C.}$ Since a 70 kilo man may produce 70 kilo. Cals./Hr., $h = 1/3600$ Cal./cc./sec. Substituting, and assuming $r_s = 10$ cm., we find $K = 0.0017$ Cal./sq. cm./sec./ $^\circ\text{C.}/\text{cm.}$ for the effective conductivity.

The bath temperature in the experiment was then raised to 36°C. so that the surface was forced to remain at that level. After about two hours the rectal temperature remained approximately constant at 37°C. Assuming that this is the new steady state and substituting once more, we find that the cylinder must now have a conductivity K^1 equal to 0.0069 cal./sq. cm./sec./ $^\circ\text{C.}/\text{cm.}$, i.e., four times the original conductivity.

In order that we may find how this new steady state was reached and follow the temperatures within the cylinder in the transition period, a solution must be found of the general differential equation:

$$\frac{d\theta}{dt} = a^2 \left[\frac{1}{r} \frac{d}{dr} \left(r \frac{d\theta}{dr} \right) \right] + b$$

where $a^2 = \frac{K^1}{s}$, $b = \frac{h}{s}$ and s is the specific heat such, that it fits the initial conditions.

We have to assume that the conductivity K^1 is constant throughout the transition (otherwise mathematical solution is beyond the capacity of the authors), that is,

that the reflex change of conductivity is completed at the start in a very short time. The solution is in terms of Bessel functions (J):

$$\theta = \theta_i - D - \frac{hr^2}{4Kl} + \sum e^{-\alpha_k^2 t} C_k J_0\left(\frac{\alpha_k r}{a}\right)$$

If the initial and final axial temps. are θ_i and θ'_i , those of the surface θ_s and θ'_s , then $D = \theta_i - \theta'_i$, and α_k is given by the roots of the equation $J_0\left(\frac{\alpha_k r_s}{a}\right) = 0$. The coefficients C_k are found by the rule

$$C_k = \frac{2}{\frac{\alpha_k r_s}{a} J_1\left(\frac{\alpha_k r_s}{a}\right)} \left\{ D + E \left(1 - \frac{4}{\left(\frac{\alpha_k r_s}{a}\right)^2} \right) \right\}$$

where $E = (\theta'_i - \theta'_s) - (\theta_i - \theta_s)$.

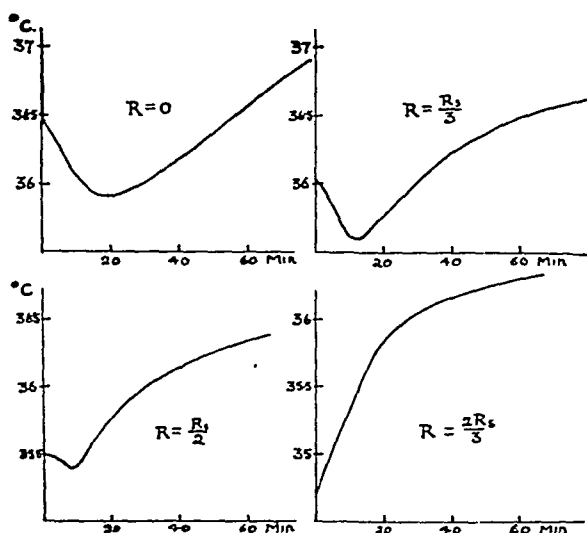


Fig. 8

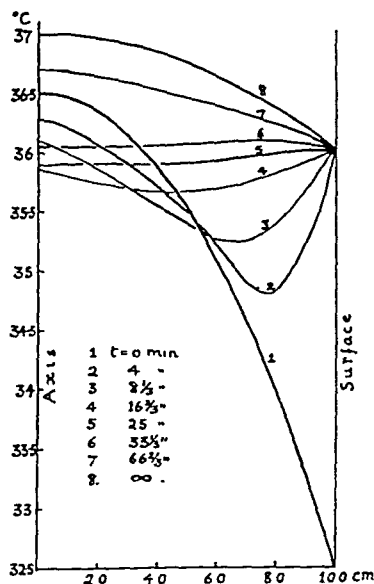


Fig. 9

Fig. 8. Time relations of thermal changes in a schematic cylinder.

Fig. 9. Thermal gradients in schematic cylinder at varying times.

Figure 8 shows how the temperature at different points in the cylinder changes with time, while figure 9 shows the temperature gradients in the cylinder at different times. The axial temperature initially falls and does not begin to rise for 20 minutes. Points nearer the surface have an initial fall of less magnitude, while those close to the surface do not show the initial paradoxical change. Whether or not the cylinder will accept heat from the bath depends on the gradient of temperature at the surface. Figure 9 shows that in this example the gradient is reversed for 25 minutes. In the actual experiment, on which the figures are based, the period of acceptance of heat by the subject was about 40 minutes. The correspondence is satisfactory considering that the choice of the radius of the cylinder, taken as 10 cm., affects the result greatly. It is difficult to estimate what value would best represent the average diameter of the "cylinders" of the immersed body. If r_s were taken as 7 cm. instead of 10 cm.,

the conductivities K and K^1 would be halved and the time to attain normal gradients become 50 minutes.

It is to be noted that a change in the thermal conductivity changes the time scale of the curves of figures 8 and 9 in the inverse ratio. The time taken to reach a steady state is longer, therefore, in a cold bath than in a hot bath.

Direct measurements of gradients in the skin up to depths of about 1 cm. had already been made under similar bath conditions (Bazett et al.), and demonstrated the presence of negative gradients. These are being reported separately.

SUMMARY

1. An insulated bathtub stirred and heated electrically, may be used as a calorimeter to measure the heat loss from the immersed surface. The surface temperature is then both constant and exactly known and the conditions of heat exchange may be measured. Such a calorimetric system has little lag, and measurements of exchange in short intervals are possible.

2. Physical regulation is able to maintain a balance between heat loss and normal heat production, when the rectal to surface temperature difference does not exceed 4°C . It is achieved by vasomotor alterations in the effective thermal conductivity which may change by a factor of about 6. In these experiments these changes were mainly of reflex origin.

3. Temperature differences exceeding 4°C . lead to an increased heat loss which exceeds normal heat production. Increased heat production (chemical regulation) is necessary but such increased heat production is accompanied by an increase in the effective thermal conductivity.

4. When the surface temperature is altered more than 1°C ., the resulting changes in rectal temperature are inadequate to indicate even the direction of change in the average temperature of the body. An approximate measure is, however, given by a formula including both surface and rectal temperatures.

5. The heat production shows a minimum when the average body temperature is at the lowest level compatible with an absence of sensations of cold. The heat production shows a better correlation with average body temperature than with rectal temperature.

6. Paradoxical changes in deep body temperature are shown to be the necessary result of the changes in the conductivity index that accompany reactions to changes in temperature. The mathematical equations controlling such changes are given.

7. Some evidence is advanced of differences in the circulatory condition and consequent conductivity index in the same subject at different seasons of the year.

REFERENCES

- BARCROFT, J., C. A. BINGER, A. V. BOCK, J. H. DOGGART, H. S. FORBES, G. HASSOP, J. C. MEAKINS AND A. C. REDFIELD. *Phil. Trans. Roy. Soc. London B* 211: 351, 1922.

BAZETT, H. C. This Journal **70**: 412, 1924.

BAZETT, H. C. AND B. MCGLONE. This Journal **82**: 415, 1927.

BORDIER, H. Arch. de Physiol. 5. **10**: 17, 1898.

BURTON, A. C. J. Nutrition **7**: 497, 1934a.

J. Nutrition **9**: 261, 1934b.

HOUGHTEN, F. C., W. W. TEAGUE, W. E. MILLER AND W. P. YANT. This Journal **88**: 386, 1929.

LEFÈVRE, J. Chaleur Animale. Paris, 1911.

LIEBERMEISTER, C. Handbuch der Pathologie und Therapie des Fiebers. Leipzig, 1875.

LILJESTRAND, G. AND R. MAGNUS. Pflüger's Arch. **193**: 527, 1922.

THE PRODUCTION OF SYMPATHIN IN RESPONSE TO PHYSIOLOGICAL STIMULI IN THE UNANESTHETIZED ANIMAL

PHILIP P. PARTINGTON

From the Department of Physiology in the Harvard Medical School

Received for publication June 1, 1936

Newton, Zwemer and Cannon (1931) observed a belated acceleration of the heart rate appearing three to four minutes after the onset of emotional excitement in unanesthetized cats with the adrenals and heart denervated. This acceleration was no longer present after the excision of the entire sympathetic system. The demonstration by Cannon and Bacq (1931) of the production of the hormone sympathin on stimulation of sympathetic nerves accounted for these observations.

Whitelaw and Snyder (1934) showed that sympathin is also liberated in the decorticate animal without adrenals during the periods of "sham rage." This condition is similar to emotional excitement in normal animals (Cannon and Britton, 1925).

In the present study an attempt has been made to show the production of sympathin in unanesthetized animals, using several methods for eliciting sympathetic activity: cold (Cannon, Querido, Britton and Bright, 1927) and hypoglycemia (Cannon, McIver and Bliss, 1924), in addition to emotional excitement. The nictitating membrane, sensitized by denervation, was used as an indicator to detect the presence of sympathin in the blood stream (Rosenblueth and Cannon, 1932).

METHOD. Cats were used. They were prepared by the aseptic removal of the right superior cervical sympathetic ganglion and section of the cervical sympathetic chain on the left side. The right adrenal was removed and the left denervated. A period of a week to ten days was allowed for the sensitization of the right nictitating membrane before any observations were made. The left membrane, only slightly sensitized to sympathin by the preganglionic denervation (Hampel, 1934), was used as a control.

To produce emotional excitement the cats were exposed to a barking, snapping dog at the door of the cage. This proved an adequate stimulus in the case of aggressive cats. In other instances the animals were tied on a board. If this alone was ineffective, release of all but one leg resulted in struggle. Insulin in the dosage of 3 units per kilogram, administered subcutaneously, was used to produce hypoglycemia. The animals went

into convulsions regularly in about 1.5 hours. The effect of cold was tested by keeping the animal for one hour in a room at 2°C.

RESULTS. Except for the periods of stimulation both membranes protruded equally over the eyes. Sympathin would be expected to induce contraction mainly on the right, highly sensitized side. These contractions were only considered positive when the visible portion of the right membrane was less than half that of the left control. No observations were made longer than one month postoperative, because of the possibility of regrowth of sympathetic fibers to the adrenals (Bacq and Dworkin, 1930).

Excitement. Exposure to a barking dog typically caused a retraction of the sensitized nictitating membrane, varying from half of the control to a mere rim. This reaction occurred in about 30 to 40 seconds and was accompanied by other signs of sympathetic activity such as erection of the hair and dilatation of the pupil. In spite of the continued presence of the dog, the membrane came back out after 2 to 3 minutes and usually protruded further than the control after the removal of the dog. At no time was there any retraction of the control membrane. One female cat which did not seem to be disturbed by the dog had no retraction of either membrane.

The results obtained by tying the cats down on an animal board were much the same as the above and had a similar time course. The animals did not usually struggle continuously for more than 2 or 3 minutes.

Hypoglycemia. Insulin in the dosage of 3 units per kilogram produced a profound lethargy within an hour, followed by nausea, vomiting, defecation, salivation, panting and dilation of the pupils. This was followed fairly promptly by generalized convulsions. During the period of other sympathetic manifestations and prior to the convulsions, the sensitized membrane retracted to a mere rim, but came back out after 2 or 3 minutes in spite of the fact that the animal went on into convulsions. It usually came further out than the control after intravenous glucose. In two rather sensitive preparations there were several periods of vomiting, each accompanied by a retraction of the membrane. In one cat that managed to get some food after the injection of the insulin, there were none of the typical manifestations of hypoglycemia, and neither membrane retracted.

Cold. Exposure to a temperature of 2°C. caused retractions of the sensitized membrane, usually 4 to 8 times during one hour. The membrane during retraction varied from about half the control to a rim and never remained in for more than 2 minutes. Retraction frequently followed a sneeze, yawn, or shivering. In one animal observed more than one month postoperative, sympathetic fibers had regrown to the control membrane. In this animal the re-innervated membrane retracted about

10 seconds before the sensitized one. The control membrane never retracted in experiments done within one month of operation.

Controls. As controls for the previous results 5 cats were prepared by sympathectomy (thoracic and abdominal) and the removal of the right superior cervical ganglion. The observations were made less than one month after the first stage of the sympathectomy because of the possibility of regrowth of the sympathetic fibers to the adrenals. Exposure to a barking dog and to a temperature of 2°C. caused no retraction of the sensitized membrane in 3 of these cats.

In the other 2 animals there was a slight retraction of the sensitized membrane to cold. Subsequent acute experiments under urethane anesthesia showed a rise in heart rate of from 8 to 12 beats per minute upon electrical stimulation of an afferent nerve. It was concluded that in these 2 cats the sympathectomy was not complete.

Discussion. Control experiments to show that the acceleration of the denervated heart after removal of the adrenals was not due to a rise of arterial pressure, increase of temperature, escape of adrenaline from the denervated adrenal medulla or cortex, nor due to substances produced by the pancreas, gastro-intestinal mucosa, semilunar ganglia, pituitary body, male gonads, thyroids, parathyroids or active skeletal muscles were done by Newton, Zwemer and Cannon (1931). In the present observations the decentralized membrane was never seen to retract while the sensitized one regularly did so. Since the only physiological agents known to affect smooth muscle, whose effects are increased by denervation, are adrenaline (excluded here) and sympathin, and since the retractions of the denervated membrane occurred under conditions favorable for the liberation of sympathin, it is concluded that the results observed were due to circulating sympathin. This is further shown by the absence of retraction of the denervated membrane after excluding circulating sympathin by total sympathectomy.

SUMMARY

After denervation of the adrenal glands various stimuli producing sympathetic activity, such as emotional excitement, hypoglycemia and cold, caused contractions of the sensitized nictitating membrane in unanesthetized cats.

Complete sympathectomy abolished these responses.

It is concluded that these contractions are due to circulating sympathin liberated in physiological conditions during sympathetic activity.

REFERENCES

- BACQ, Z. M. AND S. DWORKIN. This Journal 93: 629, 1930.
CANNON, W. B. AND Z. M. BACQ. Ibid. 96: 392, 1931.

CANNON, W. B. AND S. W. BRITTON. Ibid. 72: 283, 1925.

CANNON, W. B., M. A. McIVER AND S. W. BLISS. Ibid. 69: 46, 1924.

CANNON, W. B., A. QUERIDO, S. W. BRITTON AND E. M. BRIGHT. Ibid. 79: 466, 1927.

HAMPEL, C. W. Ibid. 111: 611, 1934.

NEWTON, H. F., R. L. ZWEMER AND W. B. CANNON. Ibid. 96: 377, 1931.

ROSENBLUETH, A. AND W. B. CANNON. Ibid. 99: 398, 1932.

WHITELAW, G. B. AND J. C. SNYDER. Ibid. 110: 247, 1934.

DIETARY AND HEMATOLOGIC STUDIES AFTER GASTRECTOMY IN THE RAT

ROBERT A. BUSSABARGER* AND FREDERIC T. JUNG

*From the Department of Physiology and Pharmacology, Northwestern University
Medical School, Chicago, Illinois*

Received for publication June 4, 1936

Work previously reported from this laboratory showed that in the white rat gastrectomy caused anemia, cessation of growth, and, eventually, a decline in weight ending in death. The uniformity of these observations led Jung (1) to conclude that the gastrectomized rat differs from the gastrectomized man, dog, and cat in becoming anemic invariably, and that its nutrition is not normal.

It was evident that further attempts should be made to evaluate various procedures that might increase the survival time of the stomachless rat. In addition certain other observations on the bone marrow, spinal cord, etc., made on this and the previous series of gastrectomized rats will be reported.

METHODS. No operated rats were used unless they had survived more than 30 days and were able to eat the moderately rough stock diets without esophageal obstruction. By gastrectomizing 225 rats we obtained 45 which were satisfactory for our purpose and which were used in the dietary experiments to be reported here.

The method given by Jung and Jones (2) for gastrectomizing rats was improved. Twenty-four hours before operation the site of the midline abdominal incision was depilated with either potassium or barium sulphide, the alkali being removed immediately after by means of boric acid. Food was then withheld until after the operation. Under ether anesthesia and aseptic precautions, laparotomy was done. The stomach was removed between light wire clamps in one of which we were careful to include the large gastro-esophageal artery. For the anastomosis, either magnesium or aluminum cannulae were used; we did not find any advantage in one metal over the other. The original cannulae were of a kind intended by Payr for the anastomosis of blood vessels. We found that a shorter form was much preferable for our purpose; the best results were obtained with cannulae 4 mm. long, flanged, and 3 mm. in internal diameter.¹ The cannula was first tied into the esophagus with 00 catgut, the ligature being

* Jessie Horton Koessler Fellow in Physiology of The Institute of Medicine of Chicago.

¹ Purchased from V. Mueller & Co., 408 S. Honore St., Chicago.

so placed as to include the large gastro-esophageal artery. Then the duodenum was drawn over the other end of the cannula and similarly tied. The abdomen was closed in two layers with catgut.

Water was placed in the cage immediately after the operation, and the rats drank freely and with evident benefit promptly after recovering from the ether. The diet for the first two weeks consisted of bread thoroughly soaked with either milk or tomato juice. During this period the rats generally lost weight. Autopsies on rats dying at various times showed that the cannulae underwent a gradual corrosion; they were rarely found after the 20th day. At about this time, also, the decline in weight generally stopped, and in some cases a rapid gain followed. The diet was then supplemented until by the 30th day the animals were on a stock diet.

Causes of death during the 30-day period were, in order of frequency, as follows: esophageal obstruction (70 per cent of deaths), local abscesses, hemorrhage, and cachexia. The deaths from obstruction would have been greatly reduced if we had discovered sooner that it was most commonly brought about by hair swallowed and caught at the anastomosis. This cause of obstruction was later completely eliminated by keeping the rats in separate cages.

Post-operative course. The post-operative growth-curves of the present series differed somewhat from those previously reported (1). Only a few rats of the present series ever regained their pre-operative weights. Generally there was a steady post-operative decline for about 2 weeks. After this, as normal eating habits were gradually resumed, the weight-curve levelled off or rose slightly to reach a plateau. The ensuing period of constant weight was often striking. Thus, rat 90 (YRF 2 male) from its 68th to its 123rd post-operative day never weighed less than 136 nor more than 149 grams; many other instances could be given. Frequently the weight of such a rat would be constant within one or two grams for a period of several days, so that the resulting curve was strikingly different from the "zig-zag" weight curve of a normal rat.

During the stage of constant weight, different animals presented different pictures. A few kept themselves clean, were active and inquisitive, and could be differentiated from normal rats only by their weight-curves and by their pale ears and eyes. No abnormalities of the teeth were ever seen. A few rats showed a curious coarsening of the hair; an example was rat 135 (PRF 1 male) whose hair (including the vibrissae) was noted after the 130th day as being long and dense. Many females (50 per cent) had persistent urinary incontinence; thus rat 99 (YRH 4 female) had periods of incontinence from the 84th to the 161st day, and again from the 201st to the 229th day, and again on the 235th day. In the intervals this rat was clean and well-nourished. Urinary incontinence was also seen occasionally (25 per cent), in the males. Priapism was frequent and per-

sistent (70 per cent) in the males; thus rat 135 (PRF 1 male) had extreme and continual priapism from the 23rd to the 50th day and again on the 148th. At these times the rat was nevertheless clean and lively.

Cause of incontinence and priapism. Genito-urinary symptoms were watched for with much interest since there was the possibility that they might be due to a degeneration of the spinal cord such as is seen in pernicious anemia. Such symptoms were frequent, as has been noted above. Attempts to obtain offspring by placing gastrectomized females with normal males, and gastrectomized males with normal females, never succeeded. Several females gastrectomized while pregnant gave birth to normal litters but refused to nurse their young. Finally four rats were sacrificed for the purpose of determining whether these abnormalities could be explained in terms of changes in the brain and cord. Neither rat 119, male, killed on the 87th day, nor rat 121, male, killed on the 101st day, had ever shown either priapism or incontinence. Rat 76 had priapism on the 13th day and had been incontinent for a few days before it was killed on its 103rd day. Rat 66 had had a period of priapism about its 74th day and had been incontinent for some time before it was killed on the 136th day. The brains and cords were instantly fixed. For the following information we are indebted to Dr. Arthur Weil (Department of Neurology) who stained the sections both for myelin and for cellular elements and examined the brains and the entire lengths of the cords. No signs of edema, inflammation, or degeneration were found; there were no histopathologic changes to indicate a subacute combined degeneration of the cord. The incontinence, priapism, and sterility of the stomachless rat cannot therefore be explained in the basis of anatomical changes in the brain or spinal cord.

Dietary procedures. Each procedure listed below was tried for periods of ten to twenty days and for several periods on not less than 2 nor more than 17 animals.

The procedures tried were:

1. Feeding stock diets I, II, and III (see table 1).
2. Feeding a bland pabulum developed in this laboratory for jejunal feeding in dogs (3).
3. Boiling the "rough" constituents of the diet.
4. Adding vitamin B (brewer's yeast) to the diet ($\frac{1}{2}$ gm. per day).
5. Adding vitamins A and D (cod liver oil) to the diet (1 gtt. per day).
6. Adding liver extract no. 343 (Lilly) to the diet (0.2 cc. per day).
7. Adding pancreatin (Merck) to the diet ($\frac{1}{4}$ gm. per day).
8. Adding banana powder to the diet (1 gm. per day).
9. Adding fresh vegetables to the diet.
10. Replacing the drinking water with iron water containing 3 grams of ferric ammonium citrate and 0.39 gram of copper sulphate per liter of tap water.

11. Replacing the drinking water with 0.4 per cent hydrochloric acid.

12. Subcutaneous injections of ferric ammonium citrate (5 cc., containing 1 mgm. of ferric ammonium citrate per cubic centimeter in 0.9 per cent sodium chloride solution) alone and together with liver extract no. 343 (0.2 cc. per day).

13. Subcutaneous injections of Phytone (1 cc. per day).²

14. Varying the daily diet.

This last procedure was apparently the best; but it did not produce satisfactory (i.e., continuous) gains in weight nor prevent decline in weight. It was noticed that nearly every marked change in diet produced a transient increase in weight.

Hematologic procedures. Blood examinations consisting of red blood cell count, hemoglobin by the Newcomer method, and determination of

TABLE 1

CONSTITUENTS	PER CENT COMPOSITION OF STOCK DIETS		
	1	2	3
Cracked yellow corn.....	60		
Ground corn.....		34	
Ground wheat.....		33	
Whole wheat flour.....			66
Powdered whole milk*.....	20	21	33
Powdered casein.....	16		
Linseed oil meal.....		7	
Alfalfa meal.....	3	2	
Vacuum dried liver meal.....		2	
Sodium chloride.....	0.5	0.5	1
Calcium carbonate.....	0.5	0.5	
	100.0	100.0	100.0

* Grateful acknowledgment is made to Nestlé's Milk Products, Inc. for supplying us gratis with "Lactogen" for this work.

mean cell diameter by direct measurement on fixed blood smears stained with Wright's stain were made every twenty days with few exceptions. Although all of the gastrectomized rats of this series developed a microcytic, hypochromic anemia which would respond to iron given orally and subcutaneously, its onset and severity were very variable in different animals. Poikilocytosis and anisocytosis increased with the severity of the anemia. It was shown by keeping 5 normal rats on a subnormal diet that the anemia of the gastrectomized rat was not due to low grade obstruction (see tables 2 and 3).

It was found that addition of vitamins A, B, and D to the diet in no way

² Generously supplied by Wilson Laboratories.

TABLE 2

SERIES	NUMBER OF DETERMINATIONS	RANGE	MEAN
Erythrocyte counts (in millions per cubic millimeter)			
Normal rats*.....	79	5.31- 9.76	7.87
Gastrectomized rats†.....	18	2.97- 9.77	5.72
Fasting rats‡.....	30	5.85-13.00	8.57
Hemoglobin (in grams per 100 cc.)			
Normal rats*.....	76	9.05-19.35	14.01
Gastrectomized rats†.....	18	4.30-14.93	8.96
Fasting rats‡.....	30	9.75-18.99	14.69
Mean diameters of erythrocytes (in micra)			
Normal rats*.....	7	6.00- 6.42	6.29
Gastrectomized rats†.....	7	4.45- 6.47	5.83
Fasting rats‡.....	0		

* Determinations made on 20 white rats of 5 different stocks.

† The counts included here were made between the 30th and 60th postoperative days on rats that lived longer than 80 days. All figures obtained at other times or after iron therapy on these rats or on rats that did not live more than 80 days are excluded.

‡ These determinations were made not less than 65 days after the normal rats had been placed on the subnormal diet.

TABLE 3

Brief, composite protocol of 5 rats kept on a subnormal diet of stock 1 to simulate weight curves of gastrectomized rats

DAYS	WEIGHT	RED BLOOD CELLS	HEMOGLOBIN
	<i>grams</i>		<i>grams</i>
0	163		
3	146	7.95	18.17
26	133	9.19	16.61
66	115	7.92	13.32
97	115	7.67	13.77
123	121	8.19	14.90
153	122	9.60	14.49
185	120	8.89	15.48
187	118	Fed ad libitum	
212	213		
227	226	9.14	16.16
247	235		

prevented the occurrence of the anemia. Addition of 0.4 per cent HCl to the diet increased rather than prevented the fall in hemoglobin. Re-

placing the drinking water of seven rats with iron water caused marked rises in hemoglobin in 5 rats, an average increase of 0.12 gram per 100 cc. per day for the 20 days on iron water, and no response, in fact, a continued fall in 2 rats. That the 5 responses were significant was shown by the fall in hemoglobin when the iron water was replaced by tap water. Ferric ammonium citrate subcutaneously caused in nearly every case an increase in red blood cell count, hemoglobin, and mean diameter. Curiously, the increase of mean diameter of the cell was proportionally more marked than the increase in Hb. In one rat (109 LFl male) the mean cell diameter increased from 4.45 to 7.55 micra. However, when the anemia was severe continuous subcutaneous iron therapy although causing some improvement could not restore the blood picture to normal. In one case orally administered liver extract (Lilly no. 343) had no effect. In two cases ferric ammonium citrate subcutaneously with orally administered liver extract (Lilly no. 343) in one case and subcutaneously administered liver extract (Lilly no. 343) in the other produced a greater remission than ferric ammonium citrate alone.

Bone marrow. The bone marrow of the femur was studied in 3 rats by Dr. F. D. Gunn, to whom we are indebted for detailed information which can only be summarized briefly here.

1. Rat 66 on its 69th day had far surpassed its preoperative weight and was in excellent condition with a red count of 8.62 million. However, its hemoglobin was only 6.64 grams, and the mean diameter of its erythrocytes was only 5.41 micra. It, therefore, had a severe microcytic and hypochromic anemia. On the 84th day daily injections of ferric ammonium citrate subcutaneously and daily administration of cod liver oil by mouth were started and continued beyond the 116th day. On this day the red count was 11.0 million, the hemoglobin 10.2 grams, and the mean diameter 5.98 micra. At this time esophageal stenosis began to develop, and the rat lost weight steadily. On the 136th day it still weighed more than at operation, but was killed for the sake of getting freshly fixed tissues. The chief differences noted between its marrow and that of normal rats was in the marked hyperplasia of the granulocytic elements, with increase of myelocytes and of immature polymorphonuclear cells and a relative paucity of erythrogenic elements. The interpretation was that the animal was suffering from a chronic infection and secondary anemia, although no evidence of infection was visible at autopsy.

2. Rat 76 on its 48th day was in excellent condition and had passed its preoperative weight. Its red count was 5.16 million, but the hemoglobin was only 4.5 grams and the mean diameter of the erythrocytes was 5.90 micra, below the lower limit of normal. It thus had a severe hypochromic and microcytic anemia. Iron and cod liver oil were given from the 52nd to the 70th day, the iron being increased gradually to the limits of tolerance. On the 83rd day the red count was 7.36 million and the mean diameter was 6.14 micra; but the hemoglobin was only 6.0 grams. Daily injections of iron were resumed, and daily additions of wheat germ to the diet were started on the 92nd day. On the 103rd day the rat was lively and its fur was clean in spite of a persistent urinary incontinence. It was killed for the sake of getting freshly fixed tissues, especially since it had both incontinence and priapism in its history. The neuropathologic findings (as stated above) proved to be negative.

In the bone marrow the erythropoietic foci were found to be extremely small and appeared to have been largely displaced by rapidly multiplying granulocytic elements. While no bacteria were found in the sections, the changes seen were typical of the hyperplasia usually found in infectious processes, although visible evidence of such a process was absent at autopsy.

3. Rat 22, which lived 320 days after operation, has too long and varied a history to be given in detail. It weighed 187 grams at operation, lost until on the 9th day it was down to 142 grams, and then started on a long course of steady gaining until on the 169th day it weighed 254 grams. It was used repeatedly in studies on the effect of iron injections. After the 282nd day it started on a steady decline which could not be stopped by iron, cod liver oil, or variations of diet. On the 320th day it weighed 159 grams; the red count was 8.75 million; hemoglobin was 9.10 grams; and the mean diameter of the erythrocytes was 3.83 micra—the most extreme microcytosis ever found by us. On this day it was killed. Examination of the bone marrow gave evidence of failure of maturation of both erythrocytic and granulocytic elements of about equal degree. The erythropoietic foci were small, though distributed fairly uniformly throughout the cellular part of the marrow. In or near each small group of undifferentiated cells there were a few normoblasts and the sinusoids contained an abundance of misshapen and various sized erythrocytes as well as a few normoblasts and many small nuclear fragments. Only occasionally were abnormal cells found in the form of megaloblasts or vacuolated erythroblasts. The megakaryocytes did not appear to be seriously altered. Altogether the changes strongly suggested “a primary anemia comparable to the pernicious anemia of the human” (Dr. Gunn).

DISCUSSION. Although the failure of all the procedures to benefit materially the nutrition of the gastrectomized rat leaves us unenlightened as to the exact nature of the deficiency, these results do show that the arrested and finally failing growth of these gastrectomized rats was not due to traumatic enteritis, to loss of appetite due to monotonous stock diets, nor to deficiencies of vitamins, iron, growth hormone from the anterior lobe of the hypophysis, pancreatic enzymes, and hydrochloric acid. Low grade obstruction due to stenosis might have been a factor in causing growth failure; but this factor was ruled out as the cause of blood changes (see table 3). Bartonella infestation was ruled out as an etiological factor in the anemia by splenectomy. The organisms were never observed, although searched for, in the course of the numerous Price-Jones counts.

The hypoplasia found in the bone marrow, the fact that iron therapy only slightly benefited the severe anemias produced by gastrectomy and the fact that iron in conjunction with liver extract produced greater remission than iron alone even though liver extract alone had no effect on the anemia suggests that absence of the stomach in the rat interferes with either the preparation or the assimilation of substances which enter into the formation of the parent erythropoietic substances of Whipple (8) which later are utilized in hemoglobin formation by the bone marrow. From these findings further experiments with liver extract and iron should

be performed. However, the results will be rather difficult to interpret until it is learned how to control better the nutritional factor.

Apparently one important function of the rat's stomach is storage of ingested food for continuous discharge to the intestines as is shown by the weight curves of normal rats compared to the more constant weight curves of gastrectomized rats. These results lead us to believe that in the rat gastrectomy not only reduces the factor of safety in digestion, as was concluded by Ivy, et al. (4) after studying gastrectomized dogs, but so markedly impairs digestion that stunted growth, secondary anemia, and early death result.

The blood findings reported here agree with those previously reported for gastrectomized rats (5) (1). Oral and subcutaneous administration of iron was effective in causing partial remissions of the anemia produced by gastrectomy. This has also been found to be true in the gastrectomized pig (6) (7). Indeed, the gastrectomized pig shows growth and blood changes qualitatively similar to the growth and blood changes of the gastrectomized rat (6) (7), except in regard to the macrocytosis induced in some rats by the injection of iron.

CONCLUSIONS

1. The nutrition of rats was seriously impaired by gastrectomy.
2. This nutritional impairment was not definitely benefited by soft diets, high vitamin diets, varied diets, and addition of pancreatic enzymes, 0.4 per cent hydrochloric acid, liver extract no. 343 (Lilly), iron, banana powder, and fresh vegetables to the diet.
3. Gastrectomized rats developed a secondary anemia of the hypochromic, microcytic type.
4. This anemia, in most cases, was improved by oral or subcutaneous administration of iron, and a relapse rapidly occurred if iron therapy was withheld.
5. Liver extract (Lilly no. 343) administered subcutaneously had no effect on the anemia in one case; but in two cases ferric ammonium citrate subcutaneously, with orally administered liver extract (Lilly no. 343) in one case and subcutaneously administered liver extract (Lilly no. 343) in the other, produced a greater remission than ferric ammonium citrate alone.
6. Absence of the stomach in the rat did not cause any changes in the spinal cord in any way suggestive of the changes seen in human pernicious anemia.
7. The bone marrow of the gastrectomized rat showed hypoplasia of erythropoietic foci with hyperplasia of granulocytic foci in two cases and hypoplasia of granulocytic foci in one case.

REFERENCES

- (1) JUNG. Thirty-sixth Annual Report of the Am. Gastroenterological Assoc., 1933.
- (2) JUNG AND JONES. Proc. Soc. Exper. Biol. and Med. **29**: 902, 1932.
- (3) SCOTT, HOLINGER AND IVY. Proc. Soc. Exper. Biol. and Med. **28**: 569, 1931.
- (4) IVY, MORGAN AND FARRELL. Surg. Gynec. and Obst. **53**: 611, 1931.
- (5) JUNG, MAISON AND HIGHSTONE. Proc. Inst. Med. Chicago, **9**: 389, 1933.
- (6) MAISON AND IVY. Proc. Soc. Exper. Biol. and Med. **31**: 554, 1934.
- (7) IVY AND BUSSABARGER. Unpublished.
- (8) WHIPPLE. J. A. M. A. **96**: 2151, 1931.

AUGMENTATION OF THE GONAD STIMULATING ACTION OF PITUITARY EXTRACTS BY INORGANIC SUBSTANCES, PARTICULARLY COPPER SALTS¹

H. L. FEVOLD, F. L. HISAW AND R. GREEP

From the Biological Laboratories, Harvard University

Received for publication June 8, 1936

Several investigators have shown that a number of substances which have no demonstrable gonad stimulating ability when given alone, will increase the action of hypophyseal gonadotropic preparations when injected with them. Evans (1933) found this to be true of the blood and urine of several mammals and attributed the augmentation to the presence of a small amount of gonadotropic substance similar to that found in the urine of pregnant women. Maxwell (1934) showed that zinc sulfate increased the action of pituitary extracts and believed that this was due to a decrease in the rate of absorption of the active material. Cassida (1935) reported that the augmenting action of the blood of cattle was a property of the formed elements rather than of the plasma. Hellbaum (1936) demonstrated the presence of augmenting substances in human urine, horse thyroid, beef liver, milk and lemon juice, while Cole and Hart (1934) and Saunders and Cole (1935, 1936) found that pregnant and non-pregnant mare serum, casein and egg albumin all enhanced the ovarian response to pituitary extracts. They, like Maxwell, believed that the increased action was due to a decrease in the rate of absorption. Friedman (1934) reported that an extract of alfalfa meal produced ovulation when injected intravenously into mature rabbits during oestrus.

We have found that aqueous pyridine extracts of dried brewer's yeast also will increase the gonadotropic activity of hypophyseal preparations in normal immature female rats. The hypophyseal extracts used were the follicle stimulating preparation (F.S.H.) and the unfractionated extract (F.S.H. plus L.H.) prepared by methods previously reported (1934). These extracts were injected into 22-day old rats in doses which alone caused very little increase in ovarian weight. The rats were injected twice daily for three days and autopsied 24 hours after the last injection. The same dosage, combined with various amounts of yeast extract, produced much greater ovarian development than that elicited by the gonadotropic

¹ Aided by a grant from the National Research Council, Committee on Problems of Sex.

preparation alone. The quantitative increase in ovarian weight was unaccompanied by any qualitative change since the F.S.H. caused the development of follicles with or without yeast while the extract containing both F.S.H. and L.H. produced luteinized ovaries in both instances (tables 1 and 2).²

The yeast extract, similar to Friedman's alfalfa extract, caused ovulation in mature rabbits. Doses of 10 to 15 grams equivalent were given intravenously at a single injection and the ovaries 48 hours later showed typical ovulation points and a number of hemorrhagic follicles. The yeast extracts were not toxic at the doses given.

The augmenting substance (or substances) in the yeast extract was very stable to heat. Boiling for several hours had no effect on its activity, nor did heating the dried extract at 100° to 110°C. impair its ability to augment gonadotropic preparations.

A sample of the yeast powder was ashed, an acid extract was made of

TABLE 1

Effect of yeast preparations on ovarian response to F.S.H.

AMOUNT OF YEAST PREPARATION (GRAM EQUIVALENT)	YEAST EXTRACT	YEAST ASH
	Ovarian weights (mgm.)	
None	16	16
1	37	35
0.5	30	33
0.25	26	24
0.1	20	21

TABLE 2

Effect of yeast preparations on ovarian response to F.S.H. plus L.H.

AMOUNT OF YEAST PREPARATION (GRAM EQUIVALENT)	YEAST EXTRACT	YEAST ASH
	Ovarian weights (mgm.)	
None	18	18
1.0	51	76
0.5	48	53
0.25	32	36
0.1	26	24

the ash and the neutralized solution tested as before. The acid-soluble ash seemed to be as active as the yeast extract in producing augmentation. It was evident therefore that at least a part of the augmenting ability was due to the inorganic constituents of the yeast.

Since qualitative tests showed that the yeast ash contained, among other things, a considerable amount of copper and iron, and since Maxwell had already reported the augmenting ability of zinc, we investigated the action of several inorganic salts. Of these, those of copper produced the greatest effect. The augmentation elicited by zinc salts was much less than that of copper while the effect of iron salts was less than that of zinc. Manganese, aluminum and calcium salts produced no or at most only slight augmentation.

The quantitative results for copper and zinc salts were very different in that copper produced greater augmentation. The qualitative results,

² The ovarian weights given in the tables in the paper are the averages for at least six animals in each case.

however, were quite similar as neither salt modified the characteristic effects of the pituitary extract with which it was combined. These observations led to a detailed comparative study of the action of salts of these two metals. The experimental procedure was the same as that used in testing the yeast extracts but in addition to normal rats, hypophysectomized rats were used. These were hypophysectomized when they were 28 days old and the experiments were started 48 hours after the operation.

Zinc sulfate, combined with F.S.H. or with F.S.H. plus L.H., increased the activity of both preparations when injected into normal 22 day old rats or into hypophysectomized rats (table 3). One milligram of zinc sulfate, injected into normal rats in combination with a constant amount of F.S.H. apparently produced a maximum augmentation, as the addition of more salt caused no further increase. Combined with F.S.H. plus L.H., the salt also produced augmented results, the increase being greater than

TABLE 3

Effect of zinc sulfate on ovarian response to F.S.H. and to F.S.H. plus L.H.

	ZnSO ₄ (mgm.)				
	None	1	3	6	10
	Ovarian weights (mgm.)				
*F.S.H.....	16	26	25	26	28
*F.S.H. plus L.H.....	19	21	45	47	
†F.S.H.....	13		22		
†F.S.H. plus L.H.....	16		46		

* Normal immature rats.

† Rats hypophysectomized 48 hours before injections were started.

when combined with F.S.H. alone. The results of similar experiments on hypophysectomized rats were comparable to those for the normal.

Copper salts increased the action of F.S.H. and of F.S.H. plus L.H. more than did zinc sulfate, when tested in normal immature rats (tables 4 and 5). Combined with the same amount of F.S.H., 0.1 mgm. copper sulfate accentuated the ovarian response approximately the same as 1.0 mgm. zinc sulfate. By increasing the amount of copper salt, the same dosage of F.S.H. increased the ovarian weight to 55 mgm. while with the zinc salt the limit apparently was reached at 26 mgm. Likewise, when added to F.S.H. plus L.H., copper salts were more effective than those of zinc. Five milligrams of copper acetate increased the effectiveness of the extract approximately 2000 per cent, $(96-15) - (19-15) / (19-15) \times 100$, while the maximum augmentation obtained with zinc salt was much less. The effects of zinc and copper salts were similar in that they were more effective when injected with a combination of both pituitary hormones than with

F.S.H. alone. Their action was also similar to yeast extract in that neither of the two kinds of salts altered the qualitative ovarian response to the extracts.

In hypophysectomized rats the addition of copper acetate to F.S.H. plus L.H. elicited an accentuated response in ovarian weight comparable to that obtained in normal immature rats (table 6). However, in such animals, copper salts did not increase the effectiveness of F.S.H., the response being the same quantitatively and qualitatively with or without copper (table 7).

TABLE 4

Effect of copper salts on the ovarian response to F.S.H. (normal rats)

	AMOUNT (MG.M.) SALT				
	None	0.1	0.5	1.0	5.0
	Ovarian weights (mgm.)				
CuSO ₄	15	25	28	45	52
Cu(C ₂ H ₃ O ₂) ₂	15	26	50	42	55
CuCl ₂	15	21	30	40	56

TABLE 5

Effect of copper salts on the ovarian response to F.S.H. plus L.H. (normal rats)

	AMOUNT (MG.M.) SALT				
	None	0.1	0.5	1.0	5.0
	Ovarian weights (mgm.)				
CuSO ₄	19	24	67	70	65
Cu(C ₂ H ₃ O ₂) ₂	19	26	46	62	96
CuCl ₂	19	23	41	58	86

TABLE 6

Effect of copper acetate (0.5 mgm.) on the response of the ovaries of hypophysectomized rats to F.S.H. plus L.H.

F.S.H. PLUS L.H. (MG.M.)	WEIGHT OF OVARIES (MG.M.), NO Cu	WEIGHT OF OVARIES (MG.M.), Cu
0.75	23	60
1.5	30	88
3.0	42	60
6.0	63	90

TABLE 7

Effect of copper acetate (0.5 mgm.) on the response of the ovaries of hypophysectomized rats to F.S.H.

F.S.H. (MG.M.)	WEIGHT OF OVARIES (MG.M.), NO Cu	WEIGHT OF OVARIES (MG.M.), Cu
0.5	17.5	18
1	21	21
2	24	25
8	32	31
16	42	40

These results bring out an important difference in the probable mechanism of augmentation produced by zinc and copper salts. The fact that copper increased the ovarian response of normal rats to F.S.H. but did not do so in hypophysectomized rats would seem to eliminate the possibility of explaining the increased action by assuming that the rate of absorption is reduced with consequent greater efficiency in the utilization of the active substance. This does not seem to be the true explanation as one would expect the salt to slow down absorption in hypophysectomized as well as in normal animals. Zinc sulfate, however, does increase the action of

F.S.H. in both types of test animals. The effect of zinc salts on F.S.H. action is quite similar to that produced by tannic acid which clearly decreases the rate of absorption. Zinc salts, therefore, as first demonstrated by Maxwell, apparently produce their effects by prolonging absorption, but it does not seem possible to explain the action of copper salts on this basis.

It was found also that intravenous injections of copper salts into mature rabbits in heat would produce ovulation within 24 to 48 hours. These results differed from those obtained by injecting pregnancy urine or pituitary extracts in only one respect, namely, that the interval between the injection of copper salts and ovulation was longer than that usually required for gonadotropic extracts. Ovulation was not observed in any case within the first twelve hours following injection. Some ovulated in 24 hours while others required a longer period. Two rabbits which received 5 mgm. of copper acetate failed to ovulate but the follicles were hemorrhagic and several were cone-shaped and appeared ready to rupture. Three received 10 mgm. of the same salt and the ovaries of each contained from 5 to 8 ovulation points. Two received 15 mgm. with results similar to those receiving 10 mgm. Zinc sulfate, chloride, or acetate in doses up to 25 mgm., did not produce ovulation or hemorrhagic follicles in seven animals which again demonstrates a difference in the effects of copper and zinc.

DISCUSSION. Augmentation resulting from a combination of gonadotropic substances and yeast extracts or inorganic salts does not seem to be of the same nature as that obtained when L.H. is combined with F.S.H. (Fevold and Hisaw, 1934). Luteinizing hormone combined with F.S.H. results in a type of augmentation which is apparently due to the two gonadotropic substances acting synergistically. This is evidenced by the fact that the augmentation is accompanied by a change in the character of the response as the ovaries contain only follicles when F.S.H. acts alone and chiefly corpora lutea when F.S.H. and L.H. act together. Yeast extract, zinc and copper salts on the other hand augment the action of F.S.H. and of F.S.H. plus L.H. without altering the qualitative action of either preparation.

Maxwell (1934) questions the duality of the pituitary gonadotropic hormones, because of the reported alteration in the ovarian response to his extracts when combined with ZnSO_4 . He intimates that our evidence for two hormones may be due to the fact that our extracts were treated with tannic acid. However, a perusal of our work will show that tannic acid does not change the qualitative response and that a difference in absorption rate is not an adequate explanation of the results (Fevold *et al.*, 1933). Likewise, using our preparations which contain both F.S.H.

and L.H. in approximately their normal balance, no change in the character of the response was observed when ZnSO_4 was added.

It seems possible that copper salts may accomplish their effects by catalyzing the synergistic action between F.S.H. and L.H. By this assumption we can explain the fact that copper salts increase the action of F.S.H. in normal rats but do not do so in hypophysectomized rats. Subminimal amounts of gonadotropic substances are apparently present in the blood of normal rats³ and the augmentation caused by copper when injected with F.S.H. may be due to the facilitation of the synergistic action of the L.H. already in the blood with the injected F.S.H. In hypophysectomized animals, the bodies of which are presumably free of the gonadotropic principles, no effect is produced by copper salt in combination with F.S.H. since no L.H. is present. If, however, L.H. is added to the F.S.H. copper salt combination the results for hypophysectomized rats are similar to those obtained for normal rats.

Ovulation in a normal, mature rabbit is generally believed to take place when the gonadotropic substances are increased to the threshold for ovulation. This is brought about apparently by nervous stimulation of the pituitary as a result of mating, or ovulation may be induced experimentally by injecting the pituitary gonad-stimulating factors. Copper salts probably cause ovulation by increasing the effective activity of the gonad-stimulating hormones present in the blood and thus the threshold for ovulation is attained. Zinc salts, which presumably cause augmentation in rats by decreasing the absorption rate, apparently have no effect on the hormones already in the blood, at least not to the extent of increasing their activity to the level required for ovulation.

SUMMARY

The gonadotropic action of follicle stimulating hormone (F.S.H.) and of F.S.H. plus luteinizing hormone (L.H.) on the ovaries of immature rats was increased when combined with yeast extract, yeast ash, zinc and copper salts. Zinc salts also augmented the action of F.S.H. and of F.S.H. plus L.H. when tested on hypophysectomized rats. Copper salts augmented the action of F.S.H. plus L.H. in hypophysectomized rats but had no effect on the activity of F.S.H. alone in such animals. Yeast extracts and copper salts caused ovulation in mature rabbits while the salts of zinc were ineffective in eliciting this response.

Zinc salts probably produce their effect by decreasing the rate of absorp-

³ The presence of gonadotropic substances in immature rats is indicated by the fact that atrophy of the gonads takes place when the pituitary is removed and also by the partial luteinization of the ovaries of a small percentage of immature rats even though "pure" F.S.H. is administered.

tion of the active material. The activity of copper salts apparently cannot be explained on this basis but may be due to a catalytic action in the synergistic interaction of F.S.H. and L.H. in ovarian development.

REFERENCES

- CASSIDA, L. E. Proc. Soc. Exper. Biol. and Med. **33**: 570, 1935.
COLE, H. H. AND G. H. HART. Proc. Soc. Exper. Biol. and Med. **32**: 370, 1934.
EVANS, H. M., M. E. SIMPSON AND P. R. AUSTIN. J. Exper. Med. **60**: 540, 1933.
FEVOLD, H. L., F. L. HISAW, A. HELLBAUM AND R. HERTZ. This Journal **104**: 710, 1933.
FEVOLD, H. L. AND F. L. HISAW. This Journal **109**: 655, 1934.
FRIEDMAN, M. H. AND G. S. FRIEDMAN. Proc. Soc. Exper. Biol. and Med. **31**: 842, 1934.
HELLBAUM, A. A. Proc. Soc. Exper. Biol. and Med. **33**: 568, 1936.
MAXWELL, L. C. This Journal **110**: 458, 1934.
SAUNDERS, F. S. AND H. H. COLE. Proc. Soc. Exper. Biol. and Med. **32**: 1476, 1935.
Proc. Soc. Exper. Biol. and Med. **33**: 505, 1936.

THE EFFECTIVENESS OF CARBON DIOXIDE IN COMBATING THE CHANGES IN VISUAL INTENSITY DISCRIMINATION PRODUCED BY OXYGEN DEFICIENCY

E. GELLHORN

From the Department of Physiology, College of Medicine, University of Illinois, Chicago

Received for publication June 12, 1936

In 1898 Mosso reported on experiments in the low pressure chamber which indicated that small concentrations of CO_2 (2 to 5 per cent) increased the resistance of his experimental subjects to lowering of the barometric pressure. Similar observations on animals were reported by Margaria (1928) and Talenti (1930) and recently Childs, Hamlin and Henderson (1935) found, on Pike's Peak, that mountain sickness could be alleviated by the inhalation of CO_2 . The only reported experiments in which, instead of lowering the barometric pressure, the partial pressure of oxygen was reduced by diluting the air with nitrogen failed to give the typical effect of CO_2 (Margaria). Since Gellhorn and Janus (1936), in their studies on the influence of oxygen deficiency on body temperature, found, in agreement with the older literature, no difference whatever between the effects of lowering the barometric pressure and diluting the air with nitrogen if the partial pressure was the same, it seemed to be of considerable interest to investigate:

1. Whether CO_2 is effective in combating the effects of oxygen deficiency if the latter is produced by air-nitrogen dilution.
2. To investigate this problem in regard to the human central nervous system where the effects due to oxygen deficiency are greatest and can be studied quantitatively, as the recent investigations of Gellhorn (1936) and Gellhorn and Spiesman (1935) show.

In the present paper the influence of 3 per cent CO_2 on the effects of oxygen deficiency on visual intensity discrimination was investigated. Fifteen experiments were carried out with uniform results. The experimental subjects inhaled the gas mixtures from Douglas bags, which, in one group of the experiments, contained 8 to 9 per cent oxygen-nitrogen mixtures, whereas in a second group, the same oxygen-nitrogen mixtures plus 3 per cent CO_2 were inhaled. As to the technique, the paper by Gellhorn (1936) may be consulted.

RESULTS. A typical result is given in figure 1, showing the effects of pure oxygen deficiency and oxygen deficiency plus 3 per cent CO_2 on the

visual intensity discrimination of two subjects. In one case (heavy line) the effect of oxygen deficiency was completely offset by 3 per cent CO_2 , although a very marked decrease was obtained in the corresponding experiment with oxygen deficiency, in which no CO_2 was inhaled. The other subject (dash line) showed a decrease in visual intensity discrimination in both instances, but the effect was considerably smaller in the presence of 3 per cent CO_2 ; and, furthermore, the original threshold was reached in the latter part of the experiment, although the subject continued to breathe $8\frac{1}{2}\text{ O}_2 + 3\text{ per cent CO}_2$.

Table 1 shows some typical experiments with five subjects in which the

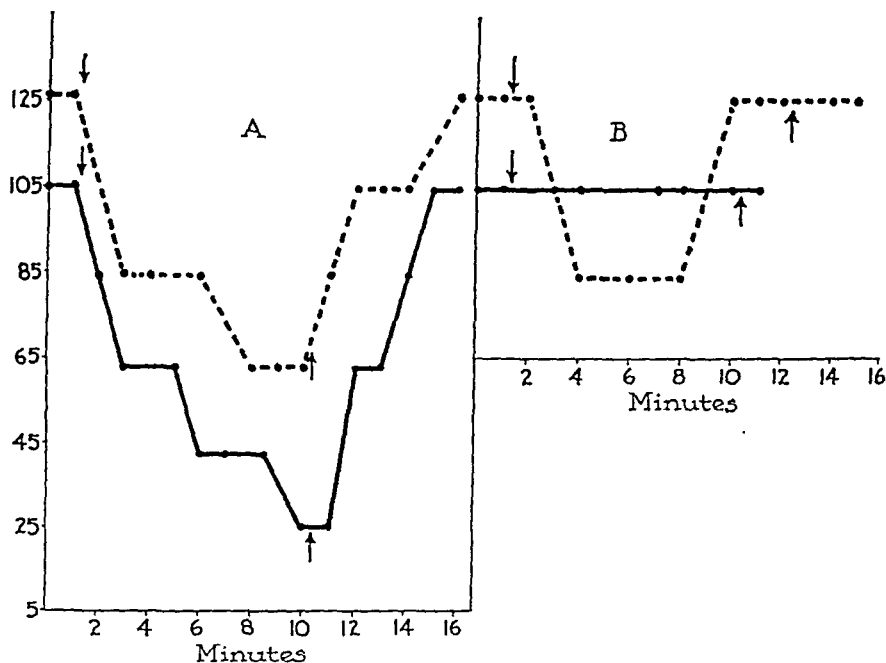


Fig. 1. Ordinate: Reciprocals of the threshold for visual intensity discrimination. Abscissa: Time in minutes. In the figure 1A and 1B $8\frac{1}{2}\text{ O}_2$ and $8\frac{1}{2}\text{ O}_2 + 3\text{ per cent CO}_2$ respectively were inhaled between the two arrows by two different subjects.

oxygen concentration varied between 8 and 9 per cent. The figures show, as a characteristic result, that the effect of oxygen deficiency is either greatly diminished or completely absent in the presence of 3 per cent CO_2 . In the former case a complete compensation is observed in the latter part of the experiment, that is ordinarily after 4 to 8 minutes. In contradistinction to this, the effect of oxygen deficiency alone on visual intensity discrimination shows a progressive character, as the figure and table, as well as the experiments of Gellhorn (1936) indicate.

A series of control experiments was carried out with 3 per cent CO_2 in air. No effect whatever was noted in these experiments. This is not

surprising in view of the fact that even with 6 per cent CO₂ the effects on visual intensity discrimination are rather slight (compare Gellhorn, 1936).

In agreement with these findings are the subjective symptoms. Practically no subjects had any complaints in the presence of CO₂, although the corresponding oxygen deficiency experiment without CO₂ led to feeling of warmth, dizziness, etc. It may, however, be emphasized that the results of oxygen deficiency are not simply due to the general interference with the

TABLE 1

The influence of CO₂ on the change in intensity discrimination produced by O₂-lack

SUBJECT	CONTROL	EXPERIMENTAL CONDITION				CONTROL		
He.	1.* 126†	9% O ₂	2. 84	5. 84	8. 63	1. 84	3. 105	6. 126
	2. 126		3. 84	7. 63	9. 63	2. 105	4. 105	7. 126
He.	1. 126	9% O ₂	1. 126	5. 84	9. 126	1. 126	3. 126	
	2. 126	+3% CO ₂	3. 84	7. 84	10. 126	2. 126		
H.	1. 147	8% O ₂	1. 147	3. 126	5. 105	1. 126	3. 147	
	2. 147		2. 147	4. 126	6. 105	2. 126	4. 147	
H.	1. 147	8% O ₂	1. 147	3. 126	5. 147	1. 147		
	2. 147	+3% CO ₂	2. 147	4. 147	6. 147	2. 147		
K.	1. 105	8% O ₂	1. 105	4. 84	6. 63	1. 105	3. 126	6. 105
	2. 105		3. 84	5. 84	7. 84	2. 126	4. 105	
K.	1. 105	8% O ₂	1. 105	4. 105	6. 105	1. 126	3. 105	
	2. 105	+3% CO ₂	3. 105	5. 105	7. 105	2. 126	4. 105	
J.	1. 126	8½% O ₂	1. 105	5. 105	8. 105	1. 126		
	2. 126		3. 84	6. 105		2. 126		
J.	1. 126	8½% O ₂	1. 126	5. 126	7. 126	1. 126		
	2. 126	+3% CO ₂	3. 126	6. 126	8. 126	2. 126		
R.	1. 105	8½% O ₂	2. 105	4. 63	6. 63	1. 105		
	2. 105		3. 63	5. 63	8. 63	2. 105		
R.	1. 105	8½% O ₂	2. 105	4. 105	7. 105	1. 105		
	2. 105	+3% CO ₂	3. 105	6. 84	8. 105	2. 105		

* Time in minutes.

† The figures represent reciprocals of the visual intensity discrimination.

well being of the experimental subject, since the same characteristic differences have been observed between the pure oxygen deficiency experiments and those in which 3 per cent CO₂ in addition to the same O₂-concentration was inhaled, although practically no subjective symptoms were reported in either of the experiments.

The observations show clearly that the beneficial effect of CO₂ on oxygen deficiency occurs in oxygen-nitrogen mixtures at normal atmospheric

pressures. The most probable interpretation seems to be that the CO₂ effect is due to:

1. The improvement of the function of the brain due to a circulatory adjustment. In favor of this assumption are the observations by Lennox and Gibbs (1932) and others cited by Gellhorn and Spiesman (1935) that CO₂ causes a dilatation of cerebral vessels, thereby improving the circulation of the brain.

2. The shift to the right in the oxygen dissociation curve of the blood, thereby increasing the rate at which oxygen is given off to the tissues.

3. The improved muscular tonus resulting even from small concentrations of CO₂ (Henderson and collaborators, 1936) which increases the venous return to the heart.

The rôle of CO₂ in circulatory adaptation to oxygen deficiency is shown further by the fact that small amounts of CO₂, which by themselves are without influence on the blood pressure of anesthetized dogs, produce, in the presence of oxygen deficiency, a distinct increase in blood pressure, as our own unpublished observations indicate.

It may be mentioned that experiments which will be published elsewhere have shown that the effect of CO₂ in alleviating the effects of oxygen deficiency was also obtained in regard to certain more purely psychic functions.

SUMMARY

The effects of breathing 8 to 9 per cent oxygen on the visual intensity discrimination in man can be either completely removed or greatly diminished by small concentrations of CO₂ (3 per cent) which in themselves have no effect on the sensory function investigated. It is believed that this effect is due to the circulatory improvement induced by CO₂ under oxygen deficiency.

REFERENCES

- CHILDS, S. B., H. HAMLIN AND Y. HENDERSON. *Nature* **135**: 457, 1935.
 GELLHORN, E. *This Journal* **115**: 679, 1936.
 GELLHORN, E. AND A. JANUS. *This Journal*, 1936.
 GELLHORN, E. AND I. SPIESMAN. *This Journal* **112**: 519, 620, 662, 1935.
 HENDERSON, Y., A. W. OUGHTERSON, L. A. GREENBERG AND C. F. SEARLE. *This Journal* **114**: 261, 1936.
 LENNOX, W. G. AND E. L. GIBBS. *J. Clin. Investigation* **11**: 1115, 1932.
 MARGARIA, R. *Arch. di Sci. biol.* **11**: 425, 1928.
 Arch. di Sci. biol. **11**: 453, 1928.
 MOSSO, A. *Life of man on the high Alps*. London, 1898.
 TALENTI, C. *Arch. di Sci. biol.* **14**: 125, 1930.

THE RÔLE OF THE DUODENAL SECRETIONS IN THE PREVENTION OF EXPERIMENTAL JEJUNAL ULCER

CHARLES M. WILHELMJ, F. T. O'BRIEN, H. H. MCCARTHY AND
FREDERICK C. HILL

*Departments of Physiology and Experimental Surgery, Creighton University School of
Medicine, Omaha, Nebraska*

Received for publication June 15, 1936

When the duodenal secretions are drained into the lower ileum and the jejunum anastomosed to the pyloric end of the stomach (Exalto, Mann-Williamson operation) chronic ulcers form in the jejunum just below the suture line in from one to four months in 95 to 100 per cent of animals (1). Mann (2) found that if the duodenum was drained into the jejunum, just below the suture line, ulcer formation was prevented. Matthews and Dragstedt (3) showed that drainage of the duodenum into the jejunum even as far as 40 cm. below the gastrojejunal anastomoses would prevent ulcer. Mann (2) and Morton (4) showed that existing experimental jejunal ulcers could often be healed by draining the duodenal secretions over the ulcer area. These observations agree in showing that jejunal ulcer can be prevented or healed if the duodenal secretions are drained into the jejunum below the gastrojejunostomy.

When the duodenal secretions are drained into the stomach the findings have not been so uniform. Mann (5) and McCann (6) found that ulcer formation was not prevented. Ivy and Fauley (7), however, found a greatly reduced incidence of ulcer (2 out of 12 animals). The findings of Mann and McCann are difficult to explain and raise the question as to why the duodenal secretions should protect the jejunum from ulcer when drained directly into the jejunum but not when drained into the stomach. Since no adequate explanation has been offered, the present experiments were planned to re-investigate the problem.

METHODS. In the work of Mann (5), McCann (6) and Ivy and Fauley (7) the duodenum was drained into the stomach by an end to side anastomosis. With this technique the stoma is often very small and it is possible that free drainage of the duodenal secretions into the stomach is interfered with. In addition the duodenum does not receive direct mechanical and chemical (acid chyme) stimulation. When mechanical and chemical stimulation is lacking we have observed that the duodenal secretions, especially the succus entericus, may be scanty in amount but can be

greatly increased by adequate stimulation. Because of these facts the method of draining the duodenum into the stomach was modified so that almost the entire duodenum was opened into the anterior wall of the stomach by a side to side anastomosis with a stoma 5 to 6 inches long. Under these conditions the duodenum virtually formed part of the anterior wall of the stomach proximal to the pyloric region, thus insuring adequate stimulation and free drainage. Often a small portion of the distal duodenum could not be used, this and the upper 6 or 8 inches of the jejunum were resected and the distal part of the jejunum anastomosed to the pylorus, end to end. Intestinal clamps and silk sutures were used because these are often cited as predisposing factors in ulcer formation.

A second series of experiments was performed in which the duodenum was drained into the jejunum a few inches below the gastrojejunostomy by a side to side anastomosis with a very long stoma.

The gastric analyses were performed with two per cent Liebig's extract test meal containing 15 mgm. of phenol red per liter (8).

RESULTS. A. *Ulcer formation.* In agreement with the work of Mann and Matthews and Dragstedt no ulcers were found in any of the four dogs in which the duodenum was drained into the jejunum below the gastrojejunal anastomosis. One dog is still living (90 weeks) and is in excellent condition (table 1).

Six dogs in which the duodenum was drained into the stomach lived from 3.3 to 39.3 weeks and of these, five were entirely negative for jejunal ulcer or jejunitis at autopsy (table 1). One dog died from acute portal thrombosis 38.6 weeks after operation. This dog was in shock for several hours before death and at autopsy a small, very superficial erosion was found in the jejunal mucosa just below the suture line. Microscopic examination showed this to involve only the superficial layer of the mucosa and to show no signs of chronicity. Considering the long duration of life after operation and the mode of death, this superficial erosion can probably be safely assumed to be a terminal event not directly related to the experimental conditions. Although the series is small, the results appear to justify the conclusion that the duodenal secretions when drained into the stomach will protect the jejunum from ulcer just as efficiently as when drained directly into the jejunum.

When the duodenum was drained into the jejunum the condition of the animals was in every way normal and the appetite remained good. When the duodenum was drained into the stomach the animals failed to remain in a normal condition, there was usually marked weight loss and general debility. Anorexia was often present, being continuous in some dogs and intermittent in others. Both of the animals which survived for over nine months showed marked weight loss; in dog VI the appetite was ravenous

throughout the entire period while in dog V a ravenous appetite was interrupted on several occasions by periods of complete anorexia.

TABLE 1

DOG NUM- BER	PERIOD OF SUR- VIVAL	ULCER AT AUTOPSY	REMARKS	TYPE OPERATION
	<i>weeks</i>			
I	3.3	None	Died. Marked anorexia and weight loss	Mann-Williamson operation with duodenal drained into the stomach
II	4.4	None	Died. Marked weight loss. Moderate anorexia for all food but meat	
III	8.4	None	Died. Appetite fair. Moderate weight loss	
IV	8.9	None	Killed with ether because of severe red mange. Appetite good. Marked weight loss	
V	39.3	None	Strangled from food in larynx. Appetite ravenous with occasional short periods of anorexia. Moderate weight loss	
VI	38.6	Small, very recent, involving only superficial layers of mucosa	Died. Portal thrombosis. Appetite ravenous. Marked weight loss	
VII	4.0	None	Killed with ether because of severe Jacksonian epileptic seizures of unknown etiology since operation. Appetite good	Mann-Williamson operation with duodenum drained into jejunum
VIII	14.4	None	Killed with ether. Hair balls in duodenal pouch and jejunum causing obstruction. Condition good until this time. Appetite good	
IX	40.4	None	Died suddenly. No cause found. Condition excellent. Appetite good	
X	90.0	No evidence of ulcer	Still living. Appetite good. Condition excellent	

B. *Gastric analysis.* In table 2 gastric analyses are shown on one dog with each type of operation.

1. *Dog V. Mann-Williamson operation with the duodenum drained into the stomach.* The resting stomach was always found to contain enormous

amounts of heavily bile stained fluid, as much as 1000 cc. were removed at times. When the acidity curve was studied with a two per cent Liebig's extract test meal, the acidity of the gastric contents due to acid secretion

TABLE 2

MEAL. MGM. CHLORIDE PER 100 cc.	GASTRIC SAMPLE, MGM. PER 100 cc.			GASTRIC CONTENTS, MGM. PER 100 cc.			GASTRIC SECRETION, MGM. PER 100 cc.			TOTAL FLUID	ACID FLUID	NON-ACID FLUID	NEUTRAL CHLORIDE NON-ACID FLUID	BILE	VOLUME OF SAMPLE, CC.	TIME, HOURS	
	Total chloride	Neutral chloride	P.S.P., per cent	Extra total chloride	Extra neutral chloride	Extra acid chloride	Total chloride	Neutral chloride	Acid chloride								
Total..... 215	261	189	79	91	52	39	433	248	185	21	7	14	3.7	+++++	35	½	Dog V. Mann- Williamson operation with duode- num drain- ed into stomach
Neutral.. 173	289	195	72	134	70	64	479	250	229	28	11	17	4.1	+++++	35	1	
Acid..... 42	332	190	62	199	83	116	523	218	305	38	19	19	4.4	+++++	36	1½	
	316	212	52	204	122	82	425	254	171	48	14	34	3.6	+++++	35	2	
	346	226	54	230	132	98	500	287	213	46	16	30	4.4	+++++	443	2½	
Total..... 232	275	191	76	99	52	47	413	217	196	24	8	16	3.3	+++	35	½	
Neutral.. 183	309	201	63	163	86	77	440	232	208	37	13	24	3.6	+++	35	1	
Acid..... 49	308	210	40	215	137	78	358	228	130	60	13	47	2.9	+++	36	1½	
	322	226	64	174	109	65	484	303	181	36	11	25	4.4	+++	573	2	
Total..... 250	288	193	83	81	49	32	476	288	188	17	5	12	4.1	+	37	½	
Neutral.. 173	305	206	76	115	75	40	479	312	167	24	7	17	4.4	+	36	1	
Acid..... 77	329	215	61	177	110	67	453	282	171	39	11	28	3.9	++	35	1½	
	342	260	54	207	166	41	450	361	89	46	7	39	4.3	+++	950	2	
Total..... 218	263	174	86	75	14	61	535	100	435	14	10	4	3.5	++	35	½	Dog X. Mann- Williamson operation with duode- num drain- ed into jejunum
Neutral.. 186	290	182	78	120	37	83	545	168	377	22	14	8	4.6	+++	35	1	
Acid..... 32	334	162	69	184	34	150	594	111	483	31	25	6	5.7	+++	35	1½	
	366	175	61	233	62	171	596	159	437	39	29	10	6.2	+++	180	2	
Total..... 222	265	162	94	56	0	56	934	0	934	6	6	0		0	35	½	
Neutral.. 176	285	173	89	87	16	71	790	146	644	11	11	0		0	35	1	
Acid..... 46	312	164	86	121	13	108	864	93	771	14	14	0		0	35	1½	
	358	184	82	176	40	136	980	222	758	18	18	0		0	852	2	
Total..... 224	307	155	74	141	28	113	543	108	435	26	19	7	4.0	trace	35	½	
Neutral.. 172	344	174	61	207	69	138	531	177	354	39	23	16	4.3	trace	35	1	
Acid..... 52	384	138	62	245	31	214	645	82	563	38	36	2	15.5	trace	34	1½	
Total..... 204	316	152	66	181	40	141	533	118	415	34	24	10	4.0	0	35	½	}
Neutral.. 170	353	134	61	228	30	198	585	77	508	39	33	6	5.0	+	34	1	
Acid..... 34	372	140	50	270	55	215	540	110	430	50	36	14	3.9	+	23	1½	
	408	158	42	322	87	235	555	150	405	58	39	19	4.6	+	51	2	

* Bile in resting stomach.

(col. 7) was always much lower than we have ever observed in any normal dog. Out of 13 half-hour samples, the single high value was 116 mgm. of acid chloride per 100 cc. of gastric contents, the remaining samples ranged from 32 to 98 mgm. per 100 cc., and during any single experiment the

values showed only small changes. The acid chloride concentration of the total secretions entering the stomach (col. 10) was also low due to the large amount of non-acid fluid of duodenal origin.

2. *Dog X. Mann-Williamson operation with the duodenum drained into the jejunum below the gastrojejunostomy.* An interesting finding in this dog was the occasional presence of bile in both the resting stomach and during a test with the Liebig's extract meal. The amount of bile and hence the amount of non-acid fluid of duodenal origin, caused pronounced changes in the gastric acidity curve. This is well illustrated by comparing the acid chloride concentration of the total fluid entering the stomach (col. 10) in experiment I in which a copious regurgitation of duodenal contents occurred, and in experiment II in which no bile was found in the gastric sample. In experiment I the values are quite normal, while in experiment II they are extremely high and resemble the values found in whole stomach pouches (8). As was previously shown these high values are due first to the fact that the non-acid fluid of intragastric origin is small in amount, and second, to the fact that absorption of water with concentration of the acid occurs. Water absorption also causes a high total chloride concentration of the total fluid entering the stomach. In the other experiments on this dog varying amounts of bile were found and the values are intermediate between the extremes found in experiments 1 and 2.

When the non-acid fluid (col. 13) is compared in dogs V and X the difference is striking, due to the difference in the amounts of non-acid fluid of intragastric and duodenal origin as pointed out in previous publications (9, 10). When there was no evidence of water absorption, the neutral chloride non-acid fluid ratio was practically the same in the two dogs.

DISCUSSION. Four of the dogs in which the duodenum was drained into the stomach lived from 8 to 39 weeks and if we discount the acute erosion in dog VI, as a terminal event associated with the cause of death, then none of the animals developed the typical ulcer seen after the Mann-Williamson operation. The recent statistical studies of Orndorff, Fauley and Ivy (11) have shown that following the Mann-Williamson operation 70 per cent of dogs will develop ulcer in 8 weeks and 100 per cent after 14 weeks. It is thus evident that when duodenal secretions are drained into the stomach, the jejunum is protected from ulcer. While this work was in progress a paper appeared by Graves (12) in which he reports a similar degree of protection and also ascribes the results of McCann to improper drainage of the duodenal contents into the stomach.

The gastric analyses on dog V show that although large amounts of acid were being secreted, the diluting and neutralizing effects of the duodenal secretions kept the *total* acidity of the gastric contents low, with one exception the values ranged from 32 to 98 mgm. of acid chloride per

100 cc. Since ulcer did not develop it is evident that there is a definite threshold value of acid necessary for ulcer formation in the jejunum, and that the above values are below the threshold level. The studies of Dragstedt (13) have shown that the threshold value for the digestion of living tissues lies between 0.10 and 0.15 per cent of *free* acid (97 to 146 mgm. of acid chloride per 100 cc.) which is considerably higher than the values for the *total* acid found in dog V.

The anorexia and loss of weight which occurred when the duodenum was drained into the stomach may be very profound. It appears likely that the anorexia is due to the distention of the stomach caused by the large amounts of duodenal secretions constantly present. The loss of weight, which may be as profound as that which occurs after the ordinary Mann-Williamson operation, was also noted by Graves (12) and by Ivy and Fauley (7). It cannot be explained solely by the anorexia since this was absent in dogs IV and VI. It is quite possible that it is due to the fact that certain essential substances in the duodenal secretions are destroyed by pepsin and hydrochloric acid and hence the organism is deprived of them just as when the duodenal secretions are drained into the lower ileum. Ivy (14) attributes it merely to insufficient intestinal digestion.

The frequent finding of bile in the gastric contents when the duodenum was drained into the jejunum shows that after the ordinary Mann-Williamson operation the succus entericus of the jejunum may at times regurgitate into the stomach and keep the acidity lower than it would otherwise be. This may have been a factor in the experiments of McCann (6) in which he found that there was no essential change in the acidity after operation.

SUMMARY

1. When the operation of surgical duodenal drainage was performed and the duodenum drained into the stomach in a manner which provided for adequate stimulation and free drainage the typical ulcers did not form in the jejunum. Gastric analysis showed that the total acidity of the gastric contents was definitely below the threshold value for the digestion of living tissues as determined by Dragstedt.

2. When the duodenum was drained into the jejunum below the gastro-jejuno-stomy ulcers did not occur. Bile was often found in the gastric contents, indicating that jejunal contents may regurgitate into the stomach after operation. The acidity curve varied with the amount of duodenal regurgitation being very high when no bile was present and lower as more regurgitation occurred.

3. Marked weight loss and anorexia were frequent when the duodenal secretions were drained into the stomach but not when they were drained into the jejunum.

REFERENCES

- (1) MANN, F. C. AND C. S. WILLIAMSON. *Ann. Surg.* 77: 409, 1923.
- (2) MANN, F. C. *Surg. Clin. N. America* 5: 753, 1925.
- (3) MATTHEWS, W. B. AND L. R. DRAGSTEDT. *Surg. Gyn. and Obstet.* 55: 265, 1932.
- (4) MORTON, C. B. *Ann. Surg.* 87: 401, 1928.
- (5) MANN, F. C. *Trans. Assn. Am. Phys.* 62: 224, 1927.
- (6) McCANN, J. C. *Arch. Surg.* 19: 600, 1929.
- (7) IVY, A. C. AND G. B. FAULEY. *Am. J. Surg.* 11: 531, 1931.
- (8) WILHELMJ, C. M., F. T. O'BRIEN AND F. C. HILL. *This Journal* 115: 5, 1936.
- (9) WILHELMJ, C. M., I. NEIGUS AND F. C. HILL. *This Journal* 107: 490, 1934.
- (10) WILHELMJ, C. M., L. C. HENRICH AND F. C. HILL. *This Journal* 110: 251, 1934.
- (11) ORNDORFF, J. R., G. B. FAULEY AND A. C. IVY. *Am. J. Digestive Dis. and Nutrition* 3: 26, 1936.
- (12) GRAVES, A. M. *Arch. Surg.* 30: 833, 1935.
- (13) DRAGSTEDT, L. R. *Ann. Surg.* 102: 563, 1935.
- (14) IVY, A. C. Personal communication.

THE ENDOMETRIAL VASCULAR BED IN RELATION TO RHYTHMIC UTERINE MOTILITY, WITH A CONSIDERATION OF THE FUNCTIONS OF THE INTERMITTENT CONTRACTIONS OF OESTRUS¹

JOSEPH FAGIN AND SAMUEL R. M. REYNOLDS

From the Department of Physiology, Long Island College of Medicine, Brooklyn, N. Y.

Received for publication June 11, 1936

The regulation of the changes taking place in the endometrial vascular bed has attracted the attention of a number of recent investigators (E. Allen, 1935) and has been shown to be under the control of the ovarian hormones. Recent work on skeletal and smooth muscle (Anrep, 1934-35) gives ample evidence, however, that muscular activity has a profound effect upon the volume flow of blood through muscles. No attempt has been made up to the present time to study the effect of myometrial activity upon the endometrial vascular bed which lies encased within a circle of uterine muscle. The purpose of the present paper is to report observations which deal with this subject.

In this study a correlation is made between the distribution and size of the small vessels and capillaries of the endometrium when there is 1, no uterine motility as in the absence of oestrin; 2, following the administration of oestrin but prior to the establishment of motility; and 3, also after treatment with oestrin but in the presence of marked rhythmic uterine motility.

METHODS AND MATERIALS. Twelve mature female rabbits of mixed stock were ovariectomized on the seventh day of pseudopregnancy. At this time the uteri are in comparable functional states. Nine days later uterine fistulae were made as described elsewhere (Reynolds, 1930; Reynolds and Friedman, 1930) and left untouched for another ten days, so allowing ample time for the hyperemic effects of the operation to subside. By the nineteenth day following ovariectomy the uterus is quiescent (Reynolds, 1931).

Four of the rabbits were left untreated whereas the other eight received a single injection of two hundred rat units of oestrin² intramuscularly on the nineteenth day. Forty-eight hours later records of motility were obtained from one uterine horn of each unanesthetized rabbit. Full anesthesia was then induced by Dial (Ciba)

¹ Aided by a grant from the Committee for Research in Problems of Sex, of the National Research Council.

² Progynon-B, generously supplied by the Schering Corporation. We are indebted to Dr. Erwin Schwenk for this hormone.

administered intramuscularly following which the abdomen was opened, the uterus gently exposed and the intestines packed out of the way in such a manner that there was minimal pressure on the large vessels of the mesometrium. The incision was then closed with clamps and the abdomen left untouched in order to minimize any disturbing influence of the above procedures. After a half-hour the wound was gently opened and 95 per cent alcohol, chilled with solid carbon dioxide (Dri-Ice), was poured quickly on a loop of relaxed uterus. The tissues froze almost instantaneously. The uterine horns used in recording motility were carefully avoided at this time, and in rabbit V-10 the uterus was contracted at the time of freezing. The solidified tissues were then excised and placed in chilled alcohol. The bottles, wrapped in cotton wool, were placed in the freezing compartment of a refrigerator and over the course of twelve to eighteen hours were thawed gradually. Throughout the next day the bottles were slowly warmed to room temperature. The tissues from the first five rabbits were cut at 12μ and stained with hemotoxylin and eosin; the tissues of the remaining seven rabbits were cut at 7μ . The results included in this report are based upon the counts made in these last seven rabbits since the thinner sections permitted very accurate studies. At least two hundred and fifty serial cross sections were obtained from each block and were studied in the manner described below.

Vascularity index. An index to the capillary and small vessel content of the endometrium was obtained in a manner similar to that described by Krogh (1929) in other tissues, except that we used no injection-mass. This technic consists of determining the number of open vessels per square millimeter in the average cross-section of endometrium. Drawings of projections of one endometrial fold were made from every tenth serial section for at least two hundred sections. The total length of uterus represented in the count was 1.4 mm. or over. It was ascertained that vessels as small as circa 4μ diameter could be regularly identified. Extensive preliminary practice with the technic showed that counts from different drawings of the same area of tissue agreed very closely, and that the same vessels were usually included from drawing to drawing, even when these were made on different days.

When counts from untreated, ovariectomized rabbits and from oestrin-treated rabbits were made it was soon learned that a certain degree of arbitrary judgment had to be exercised to select histologically comparable areas as far as freedom from edematous and disruptive changes were concerned.

The area, expressed in square millimeters, of each section of endometrium studied was determined by a planimeter, due allowance being made for the magnification of the drawing. The number of blood vessels was counted from the drawings and the number of open vessels per square millimeter of cross-sectional area thus obtained. In order to permit adequate statistical treatment of the data, twenty-five hundred to three thousand and in two instances well over four thousand vessels were counted in the total of nineteen to twenty-four sections from the various rabbits. As stated, the counts were limited to histologically comparable areas, as regards freedom from edema.

RESULTS. Summarized in table 1, in order of increasing uterine motility, are the results from uteri fixed, prepared and studied in the manner noted above.

Endometrial vascular population. It may be seen that the highest vascular counts were found in the two untreated ovariectomized rabbits having no uterine motility (V-11 and V-12). In contrast to these are the distinctly lower counts made from uteri of oestrin-injected rabbits. The

highest of the counts in these latter rabbits was from a highly active uterus (V-6), and rabbit (V-10), whose uterus was nearly as active, gave only a slightly lower count. Rabbits V-7, V-8, and V-9, also oestrin-injected, possessed uteri which were virtually inactive at the time the tissues were taken, nevertheless counts from these uteri are comparable to the counts in the two active uteri noted above. Another rabbit (V-3) not included in table 1 had a very active uterus with such extensive edema that an estimate of the density of the vascular population was impossible.

Although the number of the small vessels per square millimeter in these endometria is but a fraction of the values quoted by Krogh (1929, pp. 28-31) for skeletal muscle, the accuracy of the two methods is comparable since the percentage dispersion, indicated in table 1 for the present data, is of the same order of magnitude in both types of tissue.

TABLE 1

Correlation of rhythmic uterine motility and the density of the endometrial vascular bed (V-11 and V-12, no oestrin; all others, 48 hours after oestrin)

RABBIT	MOTILITY	NUMBER OF SECTIONS	AVERAGE NUMBER OF VESSELS PER SQUARE MILLIMETER	STANDARD DEVIATION	LIMITS OF P.E. MEAN	PER CENT DISPERSION	EDEMA
						<i>per cent</i>	
V-11	0	20	229.5	± 47.3	222.5-236.5	21	0
V-12	0	20	189.1	± 29.6	184.7-193.5	16	0
V-7	0-+	19	106.3	± 24.0	101.7-110.9	22	0
V-8	0-+	20	115.9	± 11.5	114.1-117.6	10	+
V-9	+	19	92.2	± 19.5	90.1-95.1	21	+
V-6	+++	22	125.9	± 18.9	125.2-128.6	15	++
V-10	++	24	114.6	± 17.7	112.2-117	15	++

These results betoken, therefore, a diminution of about 50 per cent in the density of the vascular population of the endometrium after the administration of oestrin. This reduction takes place prior to the initiation of motility. As the tissues become increasingly edematous, sub-epithelial hematomata are found and under the conditions of these experiments are associated with marked uterine motility. It may be noted that the diminution in number of vessels per unit area of endometrium of active uteri as contrasted with the greater number in uninjected rabbits is opposite to the situation found in skeletal muscle. In the latter, a great increase in the number of open vessels is associated with activity (Krogh, 1929). Some of the reasons for the reverse situation in the endometrium are discussed below.

Size of the endometrial vessels. The differences in average diameter of the small vessels of the untreated and treated rabbits respectively are shown in

figures 1 and 2. It will be seen in figure 1, drawn from a section of quiescent uterus from an uninjected rabbit (V-11), that the number of very small vessels is relatively great, whereas in figure 2, drawn from tissues from an oestrin-activated uterus (V-6), it will be seen that the proportion of smaller-sized vessels is low. This is one of the outstanding features of the vascular patterns noted in these studies, namely, that a state of vascular exaltation is commonly observed in endometria of oestrin-injected rabbits. Equally important is the further observation that the state of exaltation is no greater in active uteri from oestrin-treated rabbits than it is in inactive uteri, provided oestrin has been injected. The vasodilatation is, therefore, independent of myometrial activity.

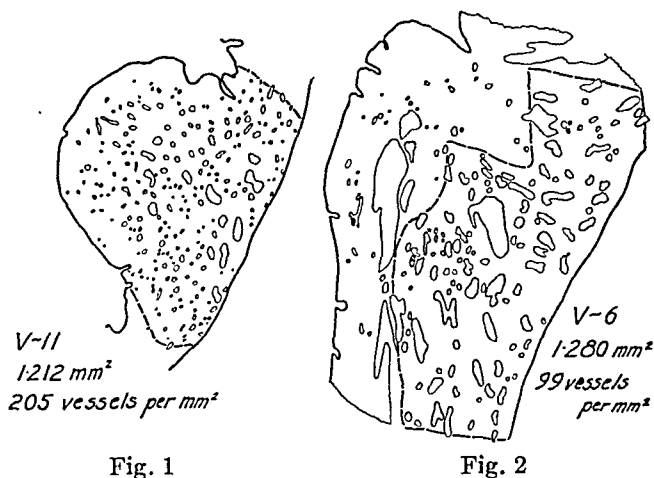


Fig. 1

Fig. 2

Fig. 1. Drawing of the vascular pattern of an endometrial fold in rabbit V-11 (originally drawn at a magnification of 131 \times). Rabbit ovariectomized three weeks; no uterine motility. Note the high vascular count, the small average size of the majority of vessels, and the evident closeness of the vessels.

Fig. 2. Drawing of the vascular pattern of a portion of an endometrial fold of rabbit V-6, 48 hours after the injection of oestrin. Ovariectomized for three weeks. Note the low vascular count, made in area at right since area at left was edematous. Note also the larger average size of the smallest vessels compared with figure 1, and the evident greater intervascular spaces as compared with figure 1. See the text for discussion.

Attention is also called to the more extensive intervascular areas that may be seen in oestrin-injected rabbits (fig. 2) than in the uninjected ones (fig. 1). This difference is attributable to an increase in the fluid matrix of the interstitial tissues of the endometrium and is a dominant factor in the reduction in density of the vascular population of the endometrium which follows the injection of oestrin. These changes also contribute to the well-known enlargement of the uterus that takes place under the influence of oestrin.

The vasodilating action of oestrin has been described in endometrial

transplants to the eye (Markee, 1932) and in the myometrium as observed through an abdominal window in rabbits (Pompen, 1933). It is found in these studies that an "initial blush" (Markee) or "peracute effect" (Pompen) occurs within a half-hour after the injection of oestrin. The initial hyperemia subsides within the next few hours but an appreciable degree of heightened color persists for the duration of the time the hormone is effective. The present results provide a basis upon which this diminution of the hyperemia may be explained since they show that the vessels become less dense in number per unit area of tissue owing to the increased permeability of the vessels upon dilatation, and the transudation of fluid which results therefrom.

Effect of myometrial contraction on the endometrial vessels. The uterus of rabbit V-10 was frozen when it was contracted, and because of this fact certain features observed in it are of interest. Table 1 shows that the number of the small blood vessels per square millimeter is similar to that found in the other oestrin-injected rabbits. In contrast to them, however, the average diameters of the vessels of V-10 are almost uniformly small, giving to the observer an impression like that which is obtained from uteri of untreated rabbits. In this case, therefore, the contraction has reduced the average size of the vessels. This is evidently accomplished by forcing ahead the blood which was in them when they were dilated. Such an interpretation is in accord with the observation of Anrep (1935) that the volume flow of blood in smooth and striated muscle is greatly increased by intermittent contractions, owing to the pumping action of the movements.

DISCUSSION. These results show that an orderly change takes place in the vascularity of the endometrium following administration of oestrin to ovariectomized rabbits. Work of others as well as our own shows that a marked vasodilatation takes place. Associated with this is an increase in capillary permeability which ultimately allows the development of edema. As a result of these changes a reduction occurs in the density of the vascular population. Inasmuch as these changes precede the initiation of rhythmic uterine motility, they are independent of it. Nevertheless intermittent contractility of the myometrium is not without effect upon the endometrial vascular bed.

In the first place, it serves to increase the volume flow of blood through the dilated endometrial vessels. In this way, freedom from congestion may be assured.

The edematous changes described above suggest one further point for consideration. Powerful rhythmic uterine contractions should serve to remove escaped plasma proteins and fluid by way of the lymphatic vessels. It will be recalled that lymph flow from most organs is facilitated by and largely depends upon intermittent activity of the parts concerned (Drinker and Field, 1932). In addition, it may be further recalled that Andersen

has shown that the lymphatic vessels of the genital tract may be easily injected only at the time of oestrus (Andersen, 1927).

In view of the foregoing considerations, it appears, therefore, that two of the principal functions served by rhythmic uterine contractility during oestrus may be to effect on the one hand an increased volume flow of blood through enlarged, hyperemic vessels and on the other, to act as an effective agent for the removal of some of the edema fluid found at this time. In this manner, rhythmic uterine motility would serve to limit and repair the vascular and fluid matrix changes induced by the direct action of oestrin.

CONCLUSIONS

1. The density of the endometrial vascular population was studied in ovariectomized rabbits, some of which were untreated, others of which were injected with oestrin. The number of open small vessels per unit area of average cross section of endometrium was correlated with the degree of uterine motility in each instance.

2. In untreated ovariectomized rabbits, the motility of the uterus was nil, and the number of open small vessels was high (circa 200 per sq. mm.). The average diameters of these vessels is small.

3. After oestrin administration, the vessels become dilated and their permeability increases. The endometrium becomes edematous, and the density of the vascular population becomes less (circa 100 per sq. mm.). These changes precede any appreciable development of rhythmic motility.

4. When the uterus exhibits rhythmic motility, the vascularity is low, the tissues edematous, and the small vessels much dilated. Sub-epithelial hematomata are also present. The relation of rhythmic motility to volume flow of blood and lymph flow in the uterus is discussed.

REFERENCES

- ALLEN, E. J. A. M. A. 104: 1901, 1935.
ANDERSEN, D. Contributions to embryology, Carnegie Institute Washington, 19: 135, 1927.
ANREP, G. V. The relation of the circulation in voluntary and plain muscle to activity. Harvey Lecture. 1934-35.
DRINKER, C. K. AND M. FIELD. Lymphatics, lymph and tissue fluid. Williams and Wilkins, Baltimore. 1933.
KROGH, A. The anatomy and physiology of capillaries. Yale Univ. Press, New Haven, 1929.
MARKEE, J. E. This Journal 100: 32, 1932.
POMPEN, A. W. M. De Invloed van Menformon op der Baarmoeder. Thesis Amsterdam, 1932.
REYNOLDS, S. R. M. This Journal 92: 420, 1930.
This Journal 97: 706, 1931.
REYNOLDS, S. R. M. AND M. H. FRIEDMAN. This Journal 94: 696, 1930.

THE EFFECT OF CERTAIN SULFUR COMPOUNDS ON THE COAGULATION OF BLOOD

J. H. STERNER AND GRACE MEDES

From the Medical Service of Lankenau Hospital and Lankenau Hospital Research Institute

Received for publication June 20, 1936

In the course of some metabolism experiments with various sulfur-containing compounds, carried out in the laboratory of the Lankenau Hospital Research Institute, confirmation of the inhibiting effect of cysteine on coagulation reported by Mueller and Sturgis (1932) was obtained. A similar effect of methionine was observed in *in vivo* but not in *in vitro* experiments. In an attempt to explain the mechanism of this inhibiting action, the following series of experiments was undertaken.

I. *In vitro experiments with whole blood.* Cysteine hydrochloride, taurine, methionine, glycine, alanine and cysteic acid, neutralized to $\text{pH } 7.0 \pm 0.1$, were added in graded amounts to whole blood giving final concentrations of from 4.4×10^{-5} to $0.18 M$, and the coagulation time was determined by the 8 mm. tube method. The results are summarized in figure 1. The abscissae represent molar concentrations of the substances, 0.4 cc. of which was added to 1 cc. of whole blood. It may be seen that a marked inhibition of coagulation occurred when taurocholic acid, taurine and cysteine were employed, whereas methionine had no effect to the limit of its solubility.

II. *In vitro experiments with the isolated components of the blood clotting system.* The components of the coagulation system were isolated by the following methods: Prothrombin was prepared from horse, rabbit and human plasmas by the method of Mellanby (1931). Fibrinogen was repeatedly salted out from horse plasma with sodium chloride (Eagle, 1934-35). The tissue factor was obtained from two sources: a, platelet suspension (Eagle, 1934-35), and b, desiccated rabbit lung (Eagle, personal communication), by drying in the Flosdorf-Mudd desiccator.¹ Calcium was added as 1 per cent CaCl_2 . The pH was controlled at 7.0 ± 0.1 with bromthymol blue as indicator. From a stock solution of cysteine hydrochloride, freshly prepared immediately before using and adjusted to pH

¹ The authors are indebted to Sharp and Dohme for generous supplies of horse blood and also to Doctor Flosdorf of the Department of Bacteriology of the University of Pennsylvania for use of their Flosdorf desiccator.

7.0 ± 0.1 with NaOH, a series of dilutions was made. In a typical experiment the following procedure and amounts of constituents were used:

Into a carefully cleaned test tube (70 mm. \times 8 mm. inside diameter) were placed 0.01 cc. of CaCl_2 solution and approximately 2 mgm. of desiccated rabbit lung or 0.02 cc. of platelet suspension. Two-tenths cubic centimeter of prothrombin solution was added, the material thoroughly mixed by shaking the tube and allowed to stand for 10 minutes (which time was found to be adequate for maximum formation of thrombin). Eight-tenths cubic centimeter of fibrinogen solution was added, the

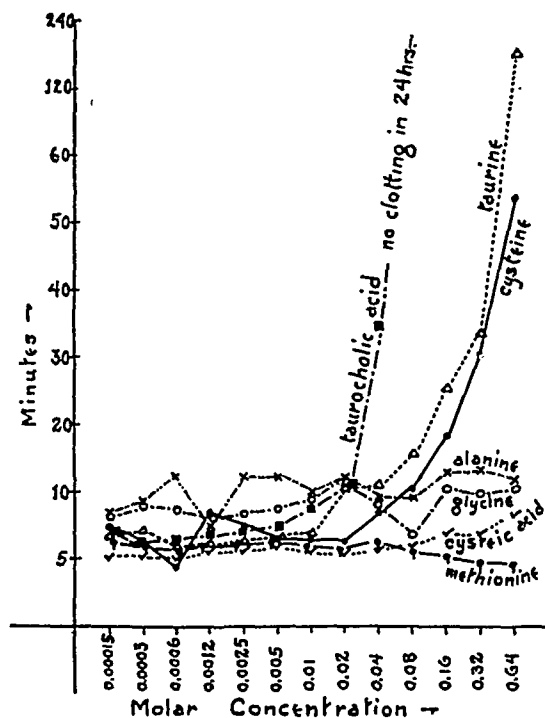


Fig. 1

Fig. 1. Effect of various compounds on the coagulation of whole blood. Abscissae, molar concentrations added, 0.4 cc. to 1 cc. of whole blood. Ordinates, time in minutes.

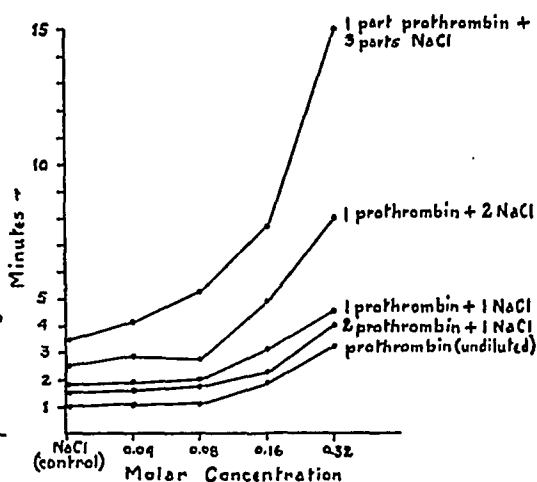


Fig. 2

Fig. 2. Effect of cysteine on coagulation time with varying concentrations of prothrombin. Abscissae, molar concentrations of cysteine. Ordinates, coagulation time in minutes.

materials mixed by a few quick shakes, and the tube examined for coagulation at 10 to 15 second intervals until it could be inverted without appreciable change in the level of the contents. The coagulation time, the time elapsing between the addition of fibrinogen and the clotting, ranged with different batches of the preparation from 30 seconds to 4 minutes, with the majority around 1 minute. The addition of cysteine at different steps in the procedure will be described below. As a control, physiological salt solution (0.85 per cent NaCl) was added in similar amounts at the corresponding time.

a. *Effect of cysteine on coagulation with varying dilutions of prothrombin.* From a solution of prothrombin which gave a coagulation time of 1 minute,

varying dilutions with physiological salt solution were made. To tubes containing calcium and tissue factor, 0.4 cc. of cysteine in concentrations from 0.32 *M* to 0.04 *M* followed by 0.2 cc. of the prothrombin in a series of dilutions in physiological salt solution were added and allowed to stand for 10 minutes before the addition of fibrinogen. The coagulation times, given in figure 2, are increasingly prolonged in the tubes containing 0.16 and 0.32 *M* cysteine, with an accentuation of the curve in those tubes with high concentrations of cysteine and more dilute prothrombin solutions.

TABLE 1

Effect on coagulation time of cysteine added before and after thrombin formation

Calcium chloride sol. (1 per cent) 0.01 cc.; platelet suspension, 0.02 cc.; prothrombin sol., 0.2 cc. cysteine hydrochloride (neutralized) in varying concentrations and 0.8 cc. fibrinogen solution were employed. In *a* cysteine was added before the prothrombin, in *b* cysteine was added 10 minutes after the prothrombin.

	CONTROL	MOLAR CONC. CYSTEINE					
		0.02	0.04	0.08	0.16	0.32	0.64
<i>a.</i> Cysteine added before thrombin formation. Coag. time (mins.).	4	4	7	24	77	167	600
<i>b.</i> Cysteine added after thrombin formation. Coag. time (mins.).		4	4	4	6	7	17

TABLE 2

Effect on coagulation time of varying periods of thrombin formation in the presence of cysteine

	COAGULATION TIME									
	Period of thrombin formation (min.)									
	5	10	15	20	30	60	120	180	240	300
Control (sec.).....	60	60	60	55	60	55	60			
0.04 <i>M</i> cysteine (sec.).....	60	55	60	60	65	60	60			
0.16 <i>M</i> cysteine (sec.).....	85	80	105	110	140	150	140			
0.32 <i>M</i> cysteine (sec.).....	370	320	310	425	425	410	355			
0.64 <i>M</i> cysteine (min.).....	117	216	246	180	326	180	100	155	190	215

b. *Effect of cysteine on coagulation when added at different phases of the coagulation process.* Table 1 shows the marked difference in the effect of cysteine when added at different phases of the coagulation process. If thrombin is allowed to form for 10 minutes before cysteine is added, the inhibition of coagulation is relatively slight compared with the considerable delay in clotting occasioned by the addition of cysteine before thrombin formation, i.e., when cysteine in 0.32 and 0.64 molar concentrations was added before thrombin formation, the coagulation times were 167 and 600

minutes, respectively, as against 7 and 17 minutes when the cysteine in the same concentrations was added after thrombin formation.

c. *Effect on coagulation time of varying periods of thrombin formation in the presence of cysteine.* Calcium, tissue factor, cysteine and prothrombin were allowed to react for varying intervals of time before the fibrinogen was added. The results, given in table 2 show that the maximal inhibition of coagulation was obtained within 5 minutes and that no additional thrombin was formed during the following 2 to 5 hours.

TABLE 3

Effect of cysteine on thrombin

Two-hundredths cubic centimeter 1 per cent CaCl_2 , 0.4 cc. prothrombin solution and approximately 2 mgm. desiccated rabbit lung, allowed to stand 10 minutes. Four-tenths cubic centimeter of 0.64 *M* cysteine was added followed by fibrinogen after varying periods. The control tube, in which the cysteine solutions were replaced with physiological salt solution, clotted in 60 seconds.

	TIME BETWEEN ADDITION OF CYSTEINE AND FIBRINOGEN (MIN.)						
	5	10	15	20	30	60	90
Coagulation time (sec.).....	210	305	335	515	570	705	645

TABLE 4

Effect of various substances on coagulation time when added to the system before and after thrombin formation

	CONTROL NaCl	MOLAR CONC.			
		0.04	0.08	0.16	0.32
a. Added before thrombin formation:					
Cysteine (sec.).....	75	95	160	230	360
Ascorbic acid (sec.).....	95	95	95	95	95
Phenosafarine (sec.).....	120	120	125	120	120
Taurine (sec.).....	90	95	95	100	95
Taurocholic acid (sec.).....	85	3 hrs.			
Taurocholic acid (sec.).....	85	3 hrs.			
b. Added after thrombin formation:					
Taurocholic acid (sec.).....	100	450	20 min.	43 min.	76 min.

d. *Effect of cysteine on thrombin for varying periods, as indicated by coagulation time.* Thrombin was allowed to form during the usual 10 minute period. Then 0.4 cc. of 0.64 *M* cysteine was added, followed by fibrinogen solution at varying intervals from 5 to 90 minutes (table 3). The slight inhibiting effect on coagulation became constant after about 30 to 60 minutes.

e. *Effect of various other substances on coagulation before and after thrombin formation.* Ascorbic acid and phenosaphranine, two compounds with reducing properties comparable to those of cysteine, produced no effect on rate of coagulation, as seen in table 4. Taurine, added to the coagulation system set up with isolated components, had no effect, contrasting with the marked effect produced when added to whole blood. Taurocholic acid, however, showed a more powerful inhibiting action than cysteine when added before thrombin formation, no clotting taking place when 0.08 *M* or

TABLE 5

Effect of cysteine on platelets (tissue factor)

Platelets obtained from horse plasma was subjected to cysteine or cysteine and CaCl_2 according to the procedure below. Control tests were made similarly with physiological salt replacing the cysteine.

(a) 0.5 cc. platelets + 1.0 cc. 0.64 *M* cysteine.

(b) 0.5 cc. platelets + 0.25 cc. of 1 per cent CaCl_2 + 1.0 cc. 0.64 *M* cysteine.

(c) 0.5 cc. platelets + 1 cc. physiological salt solution.

(d) 0.5 cc. platelets + 0.25 cc. 1 per cent CaCl_2 + 1 cc. physiological salt solution.

The platelets were recovered free from cysteine and 0.02 cc. used in coagulation tests as in the routine procedure.

	PLATELETS RECOVERED FROM			
	a	b	c	d
Coagulation time (sec.).....	70	75	75	65

TABLE 6

Effect of cysteine on fibrinogen and prothrombin as indicated by the coagulation time

(a) Fibrinogen and (b) prothrombin treated with cysteine and recovered. Coagulation time by the 8 mm. tube method.

	COAGULATION TIME			
	CONTROL NaCl	Molar conc. cysteine		
		0.16	0.32	0.64
Fibrinogen (sec.).....	40	40	50	50
Prothrombin (sec.).....	80	305	28 min.	540 min.

greater concentrations were added. It also affected the rate of clotting when added after the thrombin was formed, though the effect was far less than before its formation.

f. *Effect of cysteine on the individual components of the coagulation system.* Five-tenths cubic centimeter of platelet suspension and 1 cc. of 0.64 *M* cysteine were placed together for 15 minutes and centrifuged to recover the platelets. The latter were repeatedly washed with physiological salt solution and centrifuged until free from traces of SH. In a similar manner,

platelet suspension plus CaCl_2 solution was treated with cysteine and the platelets recovered. In table 5 it may be seen that platelets so treated were as effective as control platelets in producing coagulation.

Samples of fibrinogen were subjected to concentrations of cysteine from 0.16 to 0.64 M for 10 minutes, reprecipitated, washed free from traces of SH , and taken up in buffered physiological salt solution. The results (table 6) show no effect on the fibrinogen as indicated by the coagulation rate. No appreciable gross difference in the precipitate was detected. When prothrombin was similarly treated, however, the delay in coagulation was marked and was directly proportional to the concentrations of cysteine (table 6). There was a slightly greater yield in the control and in the lower concentrations of cysteine, but there was no apparent variance in the character of the precipitate.

A sample of rabbit plasma (citrated (0.5 per cent) blood) was added to twice the amount of 0.64 cysteine, after which the prothrombin was extracted in the manner described above. This prothrombin failed to cause coagulation in 6 hours, although the control, treated similarly with physiological salt, gave no greater yield of precipitate, but caused coagulation in the normal range of time.

As a further control prothrombin was subjected to a 0.64 M concentration of sodium hypophosphite, with no inhibition of the coagulation time over the control of 1 minute.

III. *In vivo experiments in human subjects.* a. *Methionine.* The effects produced by the ingestion and intravenous injection of methionine in 2 human subjects are shown in figure 3. For the intravenous administration, 1.31 gram of methionine was dissolved in 50 cc. of water, buffered to pH 7.0 ± 0.1 and injected into the median basilic or cephalic veins during 2 minutes. The bleeding time was determined by the method of Ivy (Ivy, Shapiro and Melnick, 1935) and the coagulation time by the 8 mm. tube method.

In both intravenous tests, depicted for the two subjects in curves *A* and *B*, the coagulation and bleeding times were definitely prolonged, although the latter was less marked in the case of *B*. The effect on the coagulation time continued almost twice as long as on the bleeding time. Curves *C* represent the effects of ingestion of 1.31 gram of methionine and curve *D*, the effects of ingestion of 3.35 grams. Following ingestion of the smaller amount, the duration of the effect was about the same for the bleeding time as for the coagulation time. After ingestion of the 3.35 grams, the curve rose higher and was more prolonged than after the ingestion of 1.31 gram.

b. *Cysteine.* One and thirty-one hundredths gram of cysteine hydrochloride in 50 cc. of water and neutralized to about pH 7.0, was injected intravenously in one fasting subject and a similar amount ingested by a second. The results are shown in figure 4. The Ivy bleeding time was increased in

both cases, rising more abruptly and falling more quickly following injection than following ingestion. The coagulation time remained within normal limits in both instances.

At intervals during the experiment, the coagulation time was determined in the presence of varying concentrations of cysteine (table 7). The usual inhibiting effect on coagulation of added cysteine was much less marked in those cases in which blood was taken at 27 minutes after ingestion and 20 minutes after injection as shown in the table, than when taken at the beginning of the experiment or at a later period. The "buffering effect"

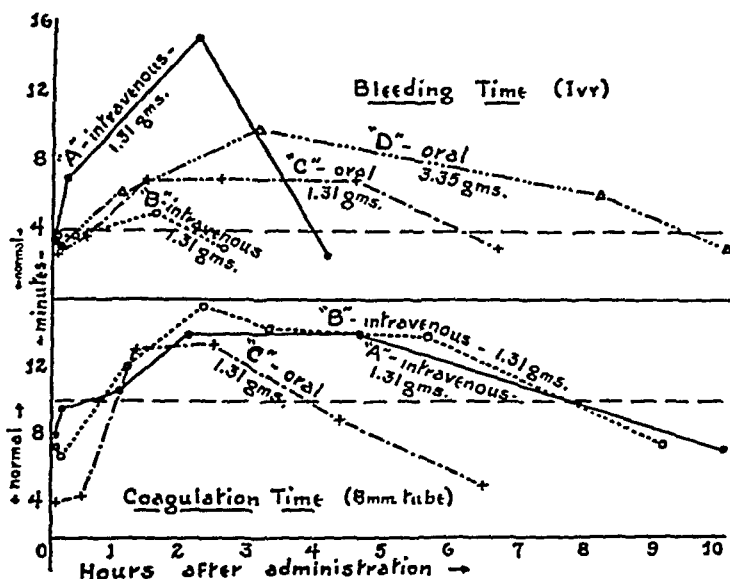


Fig. 3. Effect of ingestion and intravenous injection of methionine on bleeding time and coagulation time. Upper set of curves, Ivy bleeding time, lower set, coagulation time by the 8 mm. tube method. Curves A and B, intravenous injection of 1.31 grams methionine; C, ingestion of 1.31 grams; D, ingestion of 3.35 grams. A and D on the same subject, B and C on a second subject. Abscissae, hours after administration. Ordinates, bleeding and coagulation time, respectively.

coincided with the period of greatest prolongation of the bleeding time. (See fig. 4.)

IV. DISCUSSION. The experimental work shows that cysteine and methionine *in vivo* and cysteine *in vitro* exert a marked effect upon the mechanism of coagulation. The effect is mainly upon only one factor of the coagulation system, prothrombin. The change in the nature of this protein is proportional to the amount of cysteine added, although no loss of SH could be detected in the supernatant liquid after reprecipitating out the prothrombin. Moreover, the effect appears to be a qualitative one, for the quantity of prothrombin recovered after addition of cysteine to a prothrombin solution, is grossly equal to that of the control precipitate. The presence or absence of the cysteine, once the nature of the prothrombin

has been affected, has little or no action on the mechanism of coagulation, since fibrinogen is unaffected and thrombin only slightly inactivated.

The nature of the action on prothrombin has not been determined. Sodium hypophosphite did not inactivate prothrombin, and ascorbic acid and phenosafranine, the latter especially having oxidation-reduction potentials closely similar to that of cysteine, produced no effect when added to the reassembled isolated components. It seems hardly probable, therefore, that the effect of cysteine is due to its reducing action. Its mode of attack is certainly different from that of taurine, which caused no delay

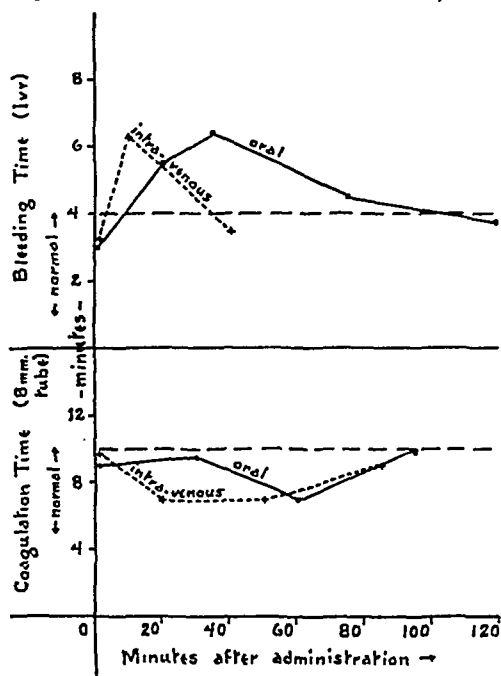


Fig. 4. Effect of ingestion and intravenous injection of cysteine on bleeding time and coagulation time. One and twenty-one hundredths grams cysteine hydrochloride (neutralized) ingested and injected in 2 different subjects. Abscissae, time in minutes after administration. Ordinates, bleeding and coagulation times, respectively.

when added similarly to the reassembled system. Taurocholic acid, on the other hand, though far more powerful in its inhibiting action, resembled cysteine in that its effect, when introduced before thrombin formation, greatly exceeded that produced when added afterwards. It is difficult to visualize any chemical action which could be possessed in common by cysteine and taurocholic acid. In view of the widespread interest recently developed in the relation of sulfhydryl to various enzyme systems and in the denaturing of proteins (Mirsky and Anson, 1934) it would be of interest to determine if a change in the number of sulfhydryl groups has occurred in the prothrombin.

These studies would confirm those of Carr and Foote suggesting that cysteine may be a responsible agent in the production of the hemorrhagic tendency in jaundice, but would indicate the defect in the formation of thrombin from prothrombin rather than in the fibrinogen factor suggested by them. In a preliminary experiment with a jaundiced patient the isolated prothrombin gave a prolonged coagulation time over a control prothrombin. But in view of the powerful inhibiting action of taurocholic acid, under conditions similar to that shown by cysteine, it would be difficult to separate these factors in jaundice.

While both the bleeding time and the coagulation time were prolonged abnormally in most instances following the administration of cysteine and methionine these deviations from normal did not always correspond.

TABLE 7

"Buffering effect" of normal blood toward coagulation-inhibiting action of cysteine:
a. After ingestion of 1.31 grams of cysteine, and b, after intravenous injection of 1.31 grams cysteine

Coagulation time expressed in minutes

	CONTROL	MOLAR CONC. CYSTEINE		
		0.08	0.16	0.32
(a) After ingestion				
1 fasting.....	9	31	47	100
2 27 min.....	9	12	16	41
3 1 hr.....	7	11	21	75
4 1 hr. 36 min.....	10	29	38	72
(b) After injection				
1 fasting.....	10	15	23	48
2 20 min.....	7	8	9	30
3 50 min.....	7	10	14	40
4 1 hr. 25 min.....	9	15	26	54

In figure 3, experiment *B*, the bleeding time following the intravenous injection of methionine is but slightly increased, in contrast to the marked variation from normal as seen in *A* under similar conditions. The corresponding coagulation time curves in these two experiments were almost identical. The results, in figure 4, show an abnormally prolonged bleeding time. In other similar experiments, not shown, the bleeding time was similarly high, and there was also a considerable increase in the coagulation time. Ivy, Shapiro and Melnick (1935) suggest that "prolonged coagulation time in jaundice is not so much an index of bleeding tendency as of liver damage." The work reported above seems to indicate that disturbance in sulfur-amino acid metabolism, without necessarily involving liver damage, may be responsible for a delayed coagulation time as well as prolonged bleeding time.

The effect of methionine *in vivo* is in sharp contrast to its behavior *in vitro*. In experiments of the latter type, there was no delay in coagulation with final concentrations of methionine up to 0.09 *M*, whereas following the administration of methionine to the human subject, with an estimated final concentration in the blood stream of 0.0016 *M*, there was a delay in the coagulation time to 12 to 16 minutes. A bleeding time, taken 10 minutes after the intravenous injection of 1.31 gram, was almost twice the value of the upper normal limit; the coagulation time, done about the same time, was normal but reached definitely elevated values after an hour. Has there been a demethylation of the S-CH₃ grouping (i.e., production of homocysteine) to bring about the coagulation delay, or has the methionine stimulated the release or production of some other substance (i.e., cysteine) to effect the change?

SUMMARY

1. Cysteine, taurine and taurocholic acid, added to whole blood, delay coagulation.
2. The action of cysteine in inhibiting coagulation is on prothrombin, preventing activation to thrombin. Tissue factor, calcium, thrombin and fibrinogen are little or not affected by it.
3. Ascorbic acid, phenosaphranine and sodium hypophosphite do not show any inhibiting effect under the conditions of these experiments.
4. Cysteine and methionine, administered orally and intravenously in the human subject, prolong both the bleeding time and the coagulation time.

REFERENCES

- CARR, J. L. AND F. S. FOOTE. Arch. Surg. 29: 277, 1934.
EAGLE, H. J. Gen. Physiol. 18: 547, 1934-35.
IVY, A. C., P. F. SHAPIRO AND P. MELNICK. Surg., Gynec. and Obstet. 60: 1, 1935.
MELLANBY, J. Proc. Roy. Soc. London B 107: 271, 1931.
MIRSKY, A. E. AND M. L. ANSON. J. Gen. Physiol. 18: 307, 1934-35.
MUELLER, J. H. AND SOMMERS, S. Sci. 75: 140, 1932.

A COMPARISON OF THREE METHODS OF MEASURING PLASMA DILUTION AFTER INTRAVENOUS SALINE INJECTION INTO NORMAL ANESTHETIZED AND FUNCTIONALLY EVISCERATED DOGS

ALLAN HEMINGWAY, DEAN A. COLLINS AND F. BERNHART

From the Department of Physiological Chemistry and Physiology of the Medical School of the University of Minnesota

Received for publication June 15, 1936

The problem of measuring changes in the plasma volume is of fundamental importance where fluid transfer within the body is involved, such as occurs in response to heat and cold, in exercise, fatigue and in hemorrhage and traumatic shock. At present the most suitable method is to use a modification of the Keith-Rowntree-Geraghty method (1916) as described by Smith (1920) in which repeated injections of a dye are made, one injection for each measurement. Objections to this method are that rapid changes of plasma volume cannot be measured since a time interval estimated by Wollheim (1928) at 40 to 80 minutes must elapse between successive measurements. With this method a sample of plasma to be used as a standard must be drawn before each dye injection and difficulties arise due to mixing time, accumulation of dye and the possibility of a variable tolerance to the dye as suggested by Lindhard (1926). An advantage in this method is that it measures the absolute plasma volume. At the present time this is perhaps the best method for measuring plasma volume changes and is superior to the methods in which concentration changes of some naturally occurring substance, as plasma protein or hemoglobin, are determined, and with the assumption that the total amount of these substances remains a constant the dilution or concentration of the plasma can be measured.

A possibility of a simple method for measuring changes in plasma volume occurs in the use of the dyes which are slowly eliminated from the vascular system. These dyes have the property of being rapidly eliminated from the vascular system during a few hours following intravenous injection but after a few days the concentration in the plasma decreases very slowly and during a two to three hour period remains practically constant. Changes in plasma volume can be determined by concentration changes of the dye. A suitable dye for this type of measurement is Wasser Blau (water soluble aniline blue), a non-toxic acid dye. This dye was found by

Wittgenstein and Krebs (1926) to persist in the plasma longer than other known dye. The elimination of this dye from dog plasma after intravenous injection has been quantitatively studied by Hemingway, Wright and Scott (1935) and it has been shown that the dye is eliminated rapidly from the plasma during the first few days following injection. About a week after injection 10 per cent of the dye remains in the plasma and is slowly excreted at a constant rate.

The purpose of the investigation reported in this paper is two-fold. In the first place the use of Wasser Blau as a dye for measurement of plasma volume changes has been investigated and, secondly, a study has been made of the ability of the vascular system to concentrate hemoglobin, plasma protein and a previously injected dye in normal and eviscerated dogs after saline injection.

From the work of Chanutin, Smith and Mendel (1924), and Calvin, Smith and Mendel (1933), it is known that after intravenous saline injections into dogs the hemoglobin is diluted and the hemoglobin returns toward the value before injection, at first rapidly and then more slowly. Two hours after injection the blood dilution lies between 0 and +20 per cent in comparison with the value before injection. The method of saline injection has been used in the experiments reported in this paper to cause changes in plasma volume. The dilution of the plasma has been measured by noting the concentration of hemoglobin, plasma protein and Wasser Blau injected one week previously. Since it is believed that the mechanism for the storing of plasma and red cells in the vascular system is in the viscera, the same experiments have been repeated on functionally eviscerated dogs.

EXPERIMENTAL METHODS. Fasting healthy dogs were anesthetized with nembutal, 35 mgm. per kilo being given to the normal dogs and 40 mgm. per kilo being given to dogs which were to be functionally eviscerated. The dogs were placed on a heated table and their rectal temperature maintained at a constant level. About one hour after receiving the anesthetic or one-half hour after the evisceration, 0.9 per cent NaCl solution heated to body temperature was allowed to flow by gravity through a needle inserted into the femoral vein. Samples of blood were withdrawn from the femoral artery at 5, 10, 15, 20, 30, 45, 60, 90 and 120 minutes after the injection period. After withdrawal about 0.25 cc. of blood was placed in a tube containing dry potassium oxalate for the hemoglobin determination and the remaining 4 to 5 cc. were discharged into 1 cc. of isosmotic potassium oxalate in a 15 cc. calibrated centrifuge tube. The tubes were centrifuged and the cell volume and total volume determined. With the operated dogs the celiac, superior mesenteric, inferior mesenteric, renal arteries and the portal and renal veins were ligated. The viscera, including the stomach, intestine, spleen, pancreas and kidneys, were removed.

Evisceration of this type has been called functional evisceration by Peterson (1934) and is applied to that type of evisceration in which all of the abdominal viscera except the liver are removed. The hepatic artery and portal vein are ligated. The operation lasted from one-half to one hour. About one and a half to two hours after giving the anesthetic the saline was injected into the operated dogs and the same procedure followed as for the unoperated dogs except that the saline injected was equal to two-thirds of the blood volume for the functionally eviscerated dogs, the unoperated dogs receiving an amount of saline equal to the blood volume.

Hemoglobin. The relative hemoglobin concentration was determined by drawing about 0.1 cc. of the blood from the tube which previously contained the potassium oxalate into a Scott (1917) pipette, i.e., a pipette with a two-way stopcock in the middle. After drawing a sample into the pipette the stopcock was turned through 180° and the blood washed from the pipette by 0.1 *N* HCl. The acidulated blood was diluted to 25 cc. and placed in a darkened bottle for 12 hours and then compared in a Duboseq colorimeter.

Plasma protein. About one-third of a cubic centimeter of oxalated plasma was drawn into a calibrated Scott pipette and washed from the pipette by about 5 cc. of distilled water into a 50 cc. centrifuge tube. The plasma proteins were precipitated by the tungstic acid reagent of Folin and Wu, centrifuged and the supernatant fluid containing the non-protein nitrogen decanted off. Concentrated sulphuric acid containing CuSO_4 was added and the proteins digested in the centrifuge tube. After digestion the digest was made alkaline and the ammonia distilled through a micro-Kjeldahl distilling apparatus, as described by Cavett (1931). The nitrogen was then determined by titration by the usual Kjeldahl method.

Wasser Blau. To 1 cc. of oxalated plasma was added 1 cc. 0.2 *N* HCl, which caused the development of the blue color of the dye in previously colorless plasma. The relative dye concentrations were determined by comparison in a Duboseq microcolorimeter using the red filter as previously described. The dye was injected into the animal one week before the experiment.

Calculation of dilution. In comparing the dilutions as measured by the hemoglobin, the value of the hemoglobin before saline injection was taken as standard and the relative plasma volume given the value of 100. The succeeding values from blood samples taken after injection of the saline were calculated from the formula

$$\text{relative volume} = 100 \frac{l_n}{l_s}$$

where l_s is the colorimeter reading of the standard and l_n the colorimeter reading of a subsequent sample.

The relative dilution of the plasma as computed from the protein dilution was computed from the formula

$$\text{relative volume (standard 100)} = 100 \frac{P_n (P_s - 1) (m - n)_s}{P_s (P_n - 1) (m - n)_n}$$

where P_s , P_n are the oxalated plasma volumes of the centrifuged blood sample, i.e., the true plasma volumes plus 1 cc. of isosmotic oxalate, of the standard and a subsequent sample. $(m - n)_s$ is the difference between the titration values of the blank m and the sample for the standard and $(m - n)_n$ is a corresponding difference for a subsequent sample.

Relative Changes of Plasma Volume after Saline Injection
Three normal dogs; saline = blood volume

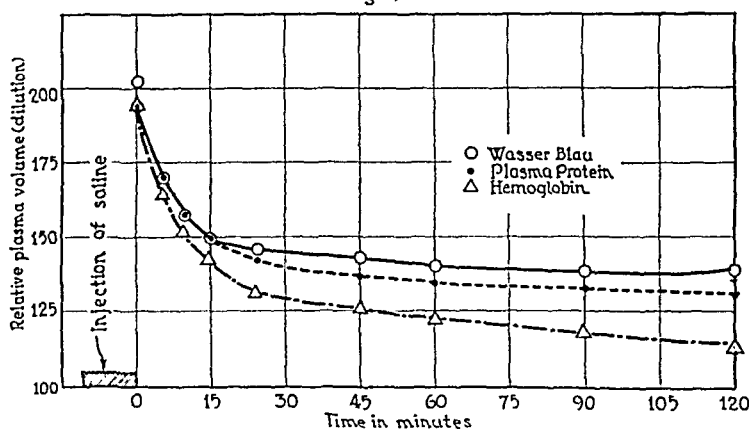


Fig. 1

The plasma volume as determined by changes in dye concentration is

$$\text{relative volume} = 100 \frac{P_n (P_s - 1)}{P_s (P_n - 1)} \cdot \frac{l_n}{l_s}$$

where P_n and P_s are oxalated plasma volumes and l_s and l_n are colorimeter readings of the standard and subsequent samples.

RESULTS. The results are given graphically in figures 1 to 3. Five minutes after an injection of saline equal to the blood volume into normal dogs anesthetized with nembutal there is a dilution of the plasma constituents of about 75 per cent (fig. 1). There is a rapid return to normal during the first 40 minutes after the injection period. During the period of from 40 to 120 minutes after injection the concentrating of the constituents is more gradual. The hemoglobin is concentrated faster than the plasma proteins while the concentrating of the dye is less than either the proteins or the hemoglobin. Two hours after the injection the blood

remains diluted as indicated by the three methods, the average dilution values being as follows: 1, hemoglobin method—15 per cent; plasma protein method—30 per cent; 3, Wasser Blau method—40 per cent. The dilution as shown by hemoglobin agrees with the results of Mendel and co-workers.

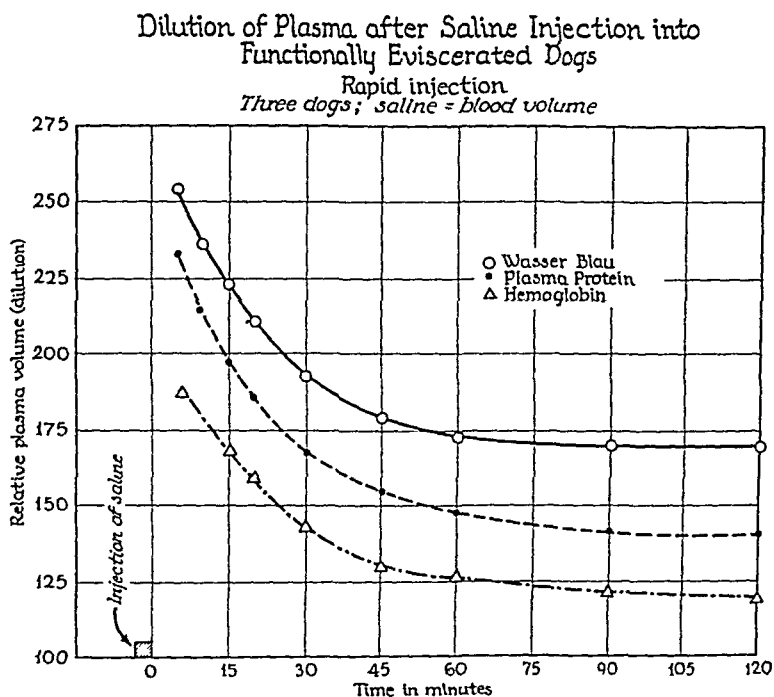


Fig. 2

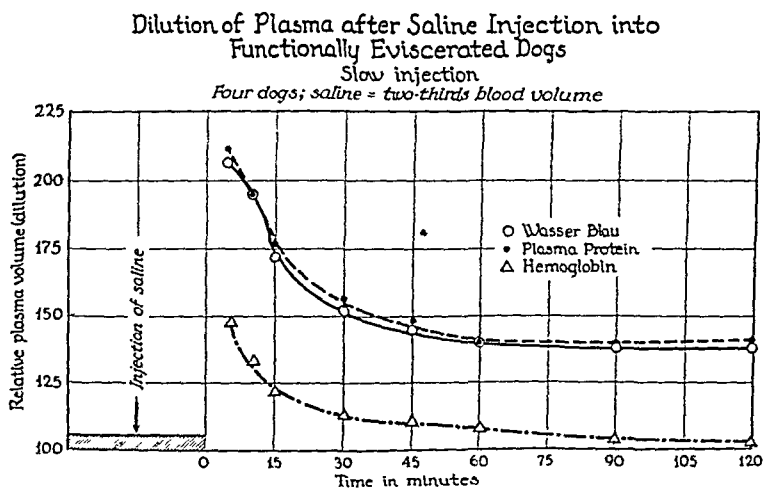


Fig. 3

In figure 2 are given the dilution values of the blood plasma after a rapid injection of saline equal to two-thirds of the blood volume into functionally eviscerated dogs. Immediately after injection the plasma dilution is

relatively great in comparison to dogs with intact viscera. In spite of the evisceration, however, the return of the plasma constituents to the normal values is rapid in the first 40 minutes and more slowly during the following 80 minutes. The dilution of the dye is greater than the dilution of the hemoglobin and the dilution of plasma proteins lies between the hemoglobin and protein. Two hours after injection the plasma volume as indicated by the Wasser Blau dilution is 70 per cent, by the plasma protein is 40 per cent and by the hemoglobin is 20 per cent.

With functionally eviscerated dogs it was expected that the dilution values of hemoglobin, plasma protein and dye would be the same after saline injection. This supposition was based on the conclusions of earlier workers who attributed to the visceral organs the property of removing or adding red cells and plasma to the circulation. The results as given in figure 2 were somewhat surprising, and it was believed that the lack of agreement between the three methods might have been due to the rapid injection of the saline, placing a strain on the vascular system by which the usual concentrating mechanisms were impaired, and protein and dye might have been lost from the blood. For these reasons it was decided to repeat the experiments using a longer injection period. Figure 3 shows the results of an injection of two-thirds of the blood volume into functionally eviscerated dogs, the injection period lasting thirty minutes. It is to be noted that after slow injection of saline the plasma protein and dye are concentrated at the same rate while the hemoglobin is concentrated faster.

Several other similar experiments were made mainly for control purposes. With a functionally eviscerated dog injection of one-twentieth of the blood volume causes a dilution of 10 to 20 per cent, which remains unchanged throughout a two-hour period. With normal dogs the concentration after slower injection resembles the concentration as indicated by the latter parts of curves of figure 1.

Discussion. These experiments are of value in establishing the part played by the viscera in experimental hydremia. The following observations from the curves are worthy of notice: 1. Immediately after saline injection the blood of eviscerated animals is diluted much more than normal animals. 2. The return toward normal concentration is rapid in both normal and eviscerated animals, the general curvature of the time-dilution curves being much the same. 3. Two hours after injection the dilution of the blood of eviscerated animals is much greater than the normal animals. The viscera act as a sort of buffer to an inflow of saline. The presence of the viscera does not affect the rate of the concentrating process but does decrease the absolute dilution.

Ludwig (1932) has postulated the existence of certain "plasma depots" in the viscera wherein the plasma can be stored out of the active circulation. Chanutin, Smith and Mendel (1924) state that the viscera play a

major rôle in water storage. Roberts and Crandall (1933) have given evidence that the portal system of the liver can remove blood from active circulation. The evidence obtained from the results given in this paper supports the view that diluted blood may be stored in the viscera but the concentrating process is a property of other tissues as well as those of the viscera.

After dilution of the plasma by saline injection into dogs with intact viscera (fig. 1) the hemoglobin returns to normal concentration faster than the proteins or the dye. For these dogs a possible explanation is that additional red cells are furnished by the spleen when the blood is diluted, causing the hemoglobin concentration to rise faster than the protein or dye concentration. The protein concentration shows a gradual return to a higher concentration during the second hour after injection while the dye concentration remains unchanged. It is possible in this case that a small amount of protein is added to the diluted plasma in an effort to restore the original concentration since it is known that protein can be rapidly formed.

The mechanism for the addition of red cells and protein supposedly lies in the viscera. With the viscera removed the concentration changes of the three constituents should be the same, that is, the curves should coincide. Figures 2 and 3 show that this does not occur. After saline injection into eviscerated dogs the dilution as shown by the hemoglobin is less and the return to a normal concentration is faster than for the plasma proteins and the dye, a result similar to that for animals with intact viscera. The lack of coincidence for the three dilution curves may be explained in two ways, namely: 1, there is a store of red cells in the bone marrow or in some other tissue which supplies red cells to the diluted blood, or 2, protein and dye leave the plasma when the blood is diluted. It is not possible to predict from the results given here which method is the effective one.

With a slow injection of saline into eviscerated and normal dogs the plasma protein changes follow closely the dye concentration changes. This suggests the formation of some sort of a molecular complex formation between the dye and protein. If the dye is bound to certain of the protein molecules the protein dilution should be the same as the dye dilution. Other evidence of the existence of such a complex is that protein precipitants which remove protein from plasma also remove the dye with the precipitated protein. This view has already been proposed by Smith (1925) who noted a parallelism between dye and protein concentration of the lymph from various parts of the body. With a rapid injection of saline into eviscerated dogs the dye dilution is greater than the protein dilution. In this case it is possible that relatively more dye than protein leaves the vascular system under the strain of the rapid injection.

Due to the uncertainty in regard to red cell reservoirs in the bone marrow and other tissues the hemoglobin changes in functionally eviscerated dogs

do not necessarily represent plasma dilutions. A better measure of plasma dilution is the protein concentration change. If it be assumed that the plasma volume varies inversely as the protein concentration after slow saline injection into normal and functionally eviscerated dogs then the dye concentration is a measure of the plasma dilution since this follows the protein concentration. The dye method, however, is not recommended for measuring rapid changes in plasma volume (fig. 3) since the dye concentration does not coincide with the protein concentration. Under usual physiological changes, however, the plasma volume changes would be of a rapidity and magnitude much less than those occurring in a rapid injection. For measuring plasma volume changes the dye method is recommended as being more convenient to use than the plasma protein method and the dye is not a natural product like the plasma protein to be added to the plasma when diluted.

The plasma volume referred to here is the total plasma volume which undergoes dilution when fluid is transferred to the plasma. This is not the "circulating plasma volume" since it has been shown by Roberts and Crandall (1933) that it is possible to have an increased total plasma volume after saline injection, without a corresponding increase in the "circulating plasma volume."

SUMMARY AND CONCLUSIONS

An amount of saline equal to the blood volume has been injected intravenously into dogs anesthetized with nembutal. The concentration changes of hemoglobin, plasma proteins and the dye Wasser Blau, injected 7 days previously, have been followed in a two-hour interval after the injection. The hemoglobin showed the least dilution after injection and returned to a dilution about 15 per cent above normal after two hours. The dye concentration and the protein concentration changes were the same in the first half hour after injection but in the latter part of the post-injection period the protein concentration increased more rapidly than the dye, perhaps due to the addition of protein to the diluted blood.

An amount of saline equal to two-thirds of the blood volume was injected slowly (during 30 min.) and rapidly (during 4 min.) into functionally eviscerated anesthetized dogs. The dilution changes as indicated by the dye and plasma proteins were the same after a slow injection but this dilution was much greater than indicated by the hemoglobin. This may indicate a storage of red cells in tissues other than the viscera. After a rapid injection the plasma dilution as indicated by the dye was the greatest and hemoglobin the least with a return to lower dilutions in the same order.

In all three sets of experiments the dilution as indicated by the dye and protein was greater than the hemoglobin. This result was unexpected for

functionally eviscerated dogs and it indicates that one or both methods for measuring plasma dilution are unreliable. Red cells may accumulate in some non-visceral reservoir or the plasma protein may leave the vascular system. If the plasma protein concentration in functionally eviscerated dogs be assumed to vary inversely as the total plasma volume, then, since the dye concentration changes coincide with the protein concentration changes, the previously injected dye method is a simple and convenient method for measuring changes in plasma volume.

REFERENCES

- CALVIN, SMITH AND MENDEL. *This Journal* **105**: 135, 1933.
CAVETT. *J. Lab. Clin. Med.* **17**: 79, 1931.
CHANUTIN, SMITH AND MENDEL. *This Journal* **68**: 444, 1924.
HEMINGWAY, SCOTT AND WRIGHT. *This Journal* **112**: 56, 1935.
KEITH, ROWNTREE AND GERAGHTY. *Arch. Int. Med.* **16**: 547, 1916.
LINDHARD, J. *This Journal* **77**: 669, 1926.
LUDWIG. *Ztschr. f. d. ges. exper. Med.* **130**: 36, 1932.
PETERSON. *Physiol. Reviews* **14**: 586, 1934.
ROBERTS AND CRANDALL. *This Journal* **106**: 423, 1933.
SCOTT, F. H. *This Journal* **44**: 298, 1917.
SMITH, H. P. *This Journal* **51**: 221, 1920.
 Bull. Johns Hopkins Hopkins Hosp. **36**: 325, 1925.
WITTGENSTEIN AND KREBS. *Pflüger's Arch.* **212**: 268, 1926.
WOLLHEIM. *Ztschr. f. klin. Med.* **108**: 463, 1928.

THE SPECIFIC GRAVITY OF THE BLOOD OF PIGEONS IN THE QUIET STATE AND DURING EMOTIONAL EXCITEMENT

L. B. NICE AND DAVID FISHMAN

From the Department of Physiology, The Ohio State University

Received for publication May 21, 1936

The physical and chemical changes in the blood of birds have been little studied. In this investigation we have measured the specific gravity of the blood of domestic pigeons in the quiet and during the excited state.

METHODS. The blood was obtained from a needle puncture of a wing vein of a pigeon in the quiet state and its specific gravity measured by the falling drop method of Barbour and Hamilton (1926). The pigeon was then excited by being restrained and teased for approximately three minutes by stimulation with a weak Faradic current. Blood was again obtained immediately thereafter from a wing vein and its specific gravity determined. The second measurement was made approximately 12 minutes after the first. Our specific gravity measurements are averages of 2 determinations in each case.

A uniform procedure was carried out in all of these experiments. Care was taken not to excite the pigeon before making the measurement in the quiet state in each one of the 28 observations. Each pigeon showed the typical signs of sympathetic stimulation during emotional excitement.

RESULTS. There was an increase in the specific gravity of the blood in each one of the 28 tests. Table 1 shows the results in ten of the observations.

The maximum, minimum, and mean values of all 28 tests were as follows: the specific gravity in the quiet state ranged from 1.0491 to 1.0538, averaging 1.0524. In the excited state it ranged from 1.0524 to 1.0555, averaging 1.0548. The increase in the specific gravity ranged from 0.0015 to 0.0036 and averaged 0.0024.

DISCUSSION. The specific gravity increase in the blood of these pigeons, during excitement was decidedly less than found under similar conditions in rabbits and cats. Nice and Katz (1935) with 20 observations on rabbits found the average specific gravity to be 1.04225 in the quiet state and 1.04890 during excitement or an average increase of 0.00665; and for 10 determinations on cats 1.03985 in the quiet state and 1.04580 during excite-

ment or an increase of 0.00595. These contrast with the average increase of 0.00240 in our pigeons.

An explanation for the increase in the specific gravity of the blood during emotional excitement is found as follows. The combined factors of a slight shift of water from the plasma into the tissues, Nice and Katz (1934); the inpouring of blood cells into the general circulation, Binet (1927); Barcroft (1930); and the addition of the products of tissue metabolism into the circulating blood causing an increase in the sugar, urea, uric acid, total and preformed creatinin, cholesterol and hemoglobin contribute to the augmentation in the specific gravity during emotional states, Nice and Katz (1934) and (1935).

TABLE 1
The specific gravity of the blood of pigeons

EXPERIMENT NUMBER	QUIET STATE	EXCITED STATE	INCREASE
1	1 0528	1 0553	0 0025
2	1 0524	1 0548	0.0024
3	1 0528	1 0553	0 0025
4	1 0526	1 0543	0 0017
5	1 0524	1 0553	0 0029
6	1 0491	1 0524	0 0033
7	1 0535	1 0553	0 0018
8	1.0524	1 0550	0 0026
9	1 0528	1 0548	0.0020
10	1 0524	1 0553	0.0029
Average.....	1.0523	1 0548	0.0025

SUMMARY

The specific gravity of the blood in each one of our 28 observations on pigeons showed an increase during emotional excitement. This increase was decidedly less than previously found under a similar condition in cats and rabbits.

REFERENCES

- BARBOUR, H. G. AND W. F. HAMILTON. J. Biol. Chem. **69**: 625, 1926.
 BARCROFT, J. J. Physiol. **68**: 375, 1930.
 BINET, L. La Physiologie de la rate. Paris, 1927.
 KATZ, H. L. AND L. B. NICE. This Journal **107**: 709, 1934.
 NICE, L. B. AND H. L. KATZ. This Journal **108**: 349, 1934.
 This Journal **113**: 205, 1935.

ACTION AND EXCITABILITY IN MAMMALIAN A FIBERS

HERBERT S. GASSER AND HARRY GRUNDFEST

*From the Laboratories of The Rockefeller Institute for Medical Research and the
Department of Physiology of Cornell University Medical College,
New York, N. Y.*

Received for publication June 22, 1936

The activity ensuing when a nerve is stimulated is made up of a chain of processes giving electrical, potential signs. The algebraic sum of these potentials is called the "action-potential." Step by step the latter term has become more comprehensive in meaning, and as the subject has developed, other terms relating to potential have been coined to denote the phenomena observed. In the interests of clarity these terms now stand in need of correlation.

Originally the term "action-potential" was used to refer to what is now called the "spike-potential." When it was realized that the process which follows the spike varies independently of it, the term "negative after-potential" was selected to differentiate the second process from the first. Finally, in line with this terminology, the positive potential, which has long been known to come at the end of activity, was called the "positive after-potential."

For descriptive purposes a general term is needed to designate the whole sequence, and special terms are required for reference to the individual events. In accord with this need, the term "action-potential" will be used in the present paper in the comprehensive sense; and the terms "spike-potential," "negative after-potential," and "positive after-potential" will be employed for particular reference to the special features of the potential accompaniment of activity. In addition, it is often convenient to have a still more comprehensive term for the sum of the events occurring in active nerve as opposed to resting nerve, without special reference to the electrical manifestations. The expression "the action" will serve very well for that purpose.

The action-potential in all kinds of fibers, in both frog and mammalian nerves, is made up of the three components mentioned above; but there are quantitative differences. In this paper, mammalian A fibers will be described and placed in comparison with previously described frog A fibers.

Frog A fibers display in a single action first the spike, then a negative after-potential starting while the spike is in progress, and finally the positive after-potential. The negative after-potential is variable in magnitude and duration, and the positive potential which appears after the ending of

the negative after-potential is very small and may be absent. Mammalian A fibers, on the other hand, present a quite different picture, largely because of the nature of the positive after-potential. The latter is sharply defined, constant in its occurrence, and in form and position varies but little from experiment to experiment, even when special precautions for the maintenance of constancy are not observed. It appears first as a well defined trough cutting into the negative after-potential, and the action-potential is thereby given a characteristic contour. What this contour is, and the possible ways in which it may be explained by potentials of component processes added together algebraically, will now be set forth in detail.

The spike-potential. The whole train of events occurring in the action is initiated by the spike process. In order to get the form of the latter, the best procedure is to record spike-potentials in single fibers. When this is done, a result is obtained that necessitates some revision of the duration, 0.6 msec., which was given following the original observations of the mammalian spike with the cathode ray oscillograph. The value mentioned was obtained in experiments in which a lead was taken from the stimulating cathode. In that way, error arising from temporal dispersion was eliminated (except for a negligible error arising from conduction from one side to the other of the silver wire which served as the electrode (Blair and Erlanger, 1933, p. 531)); but the utilization period of the shock was included with the spike duration, and the method was such as to measure the longest components—if there be variation in the duration of mammalian A spikes, similar to that described by Blair and Erlanger (1933) for frog fibers. Records of single spikes show that for one or the other of the foregoing reasons, 0.6 msec. is too large a value.

For single-fiber records, spinal roots of the cat were used. This preparation has two advantages, both attributable to the absence of a sheath. Small strands may be separated out, so that the potential in an active fiber is shunted by only a small number of inactive fibers; and the shock artifact is reduced to a minimum. Stimuli applied at threshold cause the most irritable fiber in the nerve to respond in isolation in a percentage of trials sufficient to permit obtaining numerous single responses in a series of photographs. Records made in this way show regularly that the spike duration—and by this is meant the major part of the spike, exclusive of the tail—is very close to 0.4 msec. at 37.5 degrees C. (fig. 1). Readings of 0.4 to 0.43 msec. are obtained in the best preparations, there being uncertainty as to the precise figure, because of the impossibility of selecting precise points between which to measure. Of this duration, the period of rising potential takes up one-third, and the falling potential two-thirds of the time.

On the basis of this value it is interesting to compare the wave lengths in

roots of the cat and the bullfrog. The velocity in a threshold fiber in the cat would be 90 m.p.s., and the wave length, therefore, 3.6 cm. In the bullfrog the velocity would be 40 m.p.s., with a corresponding spike duration of 0.9 msec., giving again a wave length of 3.6 cm.

The after-potentials were observed usually on the saphenous or the phrenic nerve of the cat. Other nerves were examined only to prove that those named are typical of mammalian A fibers in general. Temporal dispersion, unless the spike height is being compared with the magnitude of the after-potentials, is not important in after-potential studies. Therefore about two centimeters of conduction were allowed to keep the action-potential conveniently free from the shock artifact. When the correct height of the spike was important, the lead was made close to the cathode.

For after-potentials, a direct current amplifier was used. The direct current amplifier is being improved continually in the interest of smaller distributed capacity, smaller drift, and a lower noise level. That used in this investigation is much better than the one mentioned in a previous paper (Gasser, 1935), but falls short of the newest model. Its calibration curve, given in figure 2, still shows somewhat more capacity effect than the A. C. amplifier, and therefore A. C. amplification was used for the measurement of spikes. The nerves were mounted in a moist chamber at 37 degrees C. and supplied with oxygen, or in some cases with oxygen containing 5 per cent of carbon dioxide.

The negative after-potential, when first visible, is completely fused with the spike. No suggestion of a rising phase, such as is often encountered in frog nerve (Gasser and Graham, 1932) is seen; and in nerves successfully rendered monophasic by the application of cocaine at the indifferent lead, spike and after-potential fuse without evidence of discontinuity (fig. 5 a). That there is discontinuity, however, can readily be brought out by painting the nerve with 1:250,000 veratrine. Without change in the spike, the negative after-potential begins to increase in size, principally by being prolonged. Starting at the junction of the spike and the after-potential, the action-potential subsides more slowly in each successive record as veratrinization progresses. Thus, when successive records are superimposed, the negative after-potentials appear to pivot about the point at which they differentiate from the spike (fig. 3).

Veratrinization also demonstrates that the decremental form of the negative after-potential is not a necessary quality of the process. In analogy with mammalian C fibers and frog A fibers, the negative after-potential should have a rising phase of its own and appear with a convex contour. Both of these qualities appear when the nerve is veratrinized. As veratrinization proceeds, rising phases develop spontaneously, especially if the nerve is in an atmosphere containing 5 per cent of CO₂, instead of in pure oxygen. But to bring out the development strikingly, the

response should be set up during the positive after-potential following a tetanus, in imitation of the procedure which has been found to bring it out in frog fibers (Gasser and Graham, 1932). The inset in figure 3 shows a growing after-potential set up in this way.

Because of overlapping with the spike, no definite value can be given for the magnitude of the negative after-potential. At the point where the slope in the decline of the action-potential begins sharply to decrease—that is, where the after-potential begins definitely to contribute to the sum of the potentials—the potential of the action is about 5–6 per cent of that obtaining at the crest of the spike. From this point the potential declines along a decremental curve. It reaches zero normally at about 15 msec. and is then continued by a deflection on the positive side of zero, which reaches a maximum at about 30 msec. and continues positive to about 70 msec. These durations are based on experiments which will be described in the latter part of this paper. Isolated nerves mounted in air show potentials which follow the same course, but the cycle is faster, so that zero potential may be reached at 12 msec. instead of at 15 to 18 msec.

Compared with the potential of the spike, the magnitude of the positive after-potential is small. When the latter reaches its maximum, a representative value would be 20 to 25 μ v. The corresponding spike would reach 12 mv. at its crest; hence the after-potential amounts to only about 0.2 per cent of the spike-potential. Variations from 0.1 per cent to 0.4 per cent are encountered.

In isolated nerves the action-potential may end with the positive dip, but that mode of ending does not describe all the cases seen. There is often a suggestion of ensuing negativity. In order to describe this negativity, it is necessary to divide nerves into two groups: those which are rhythmic and those which are non-rhythmic. Nerves freshly mounted in air or oxygen—particularly phrenic nerves—in addition to the negative and positive phases already described, are apt to show a second negative potential, and even a third and a fourth one. In other words, the play between negativity and positivity behaves like a damped oscillation (fig. 4). Preparations which show this rhythmicity well are also subject to spontaneous discharging in their fibers, and this discharge increases during the negative phases and decreases during the positive ones. Such a variation in the discharge would by itself produce the appearance of rhythmicity, and hence the question is raised as to the extent to which the discharge is the cause of the rhythm. To this question the most probable answer is that the rhythm is attributable primarily to the original action. On this basis, the changes in the spontaneous discharge are readily explained: the increase by the supernormality which goes with negative after-potential, and the decrease by the subnormality which goes with positive after-potential; and without the assumption that the after-potential is rhythmic,

the variation in the spontaneous discharge would not be accounted for. Further difficulty would then also be presented in that many of the negative waves do not look like pure spike aggregates, and that often there are not enough spikes in the negative phases to permit accounting for the negativity as negative after-potential connected with the fresh discharge. On the other hand, even though the rhythm is initiated by the original action, it must be augmented and perpetuated by the resulting effect on the discharge. The bursts of spikes at the negative crests would accumulate a tendency for the potential to turn to positive again. In the negativity following this positivity, discharge would again occur, and so the cycle would continue.

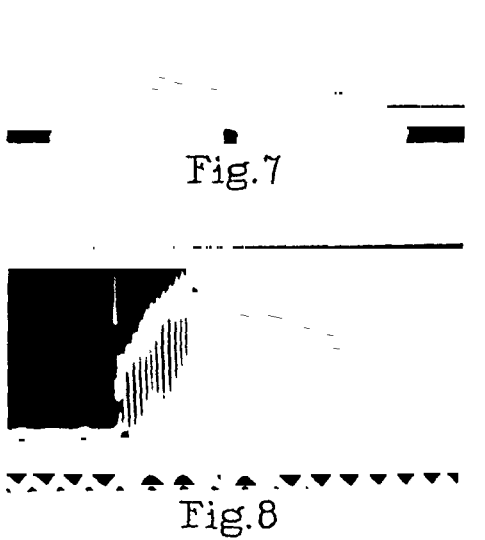
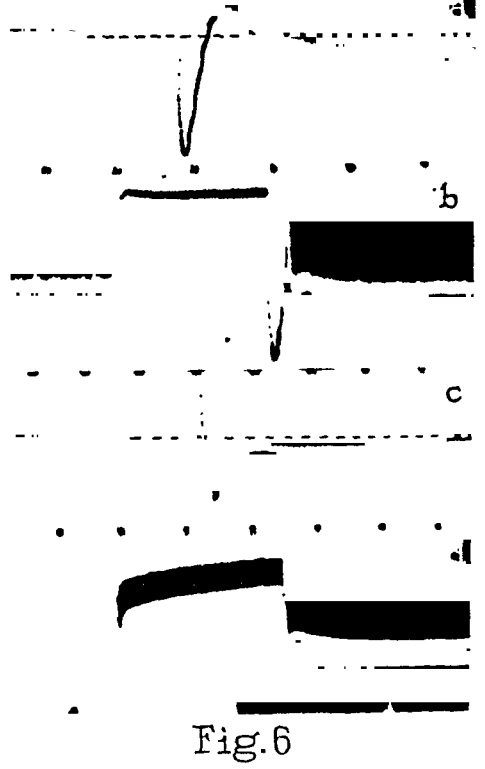
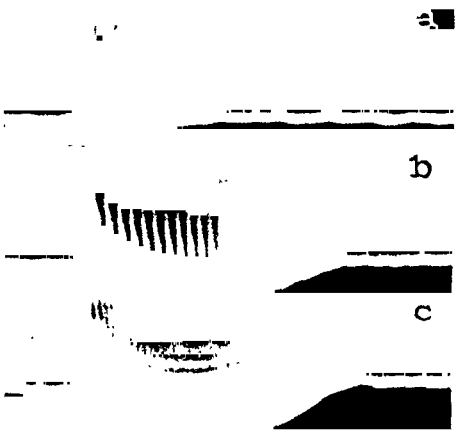
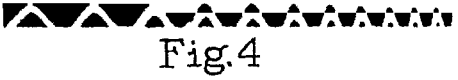
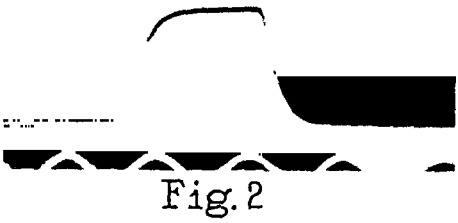
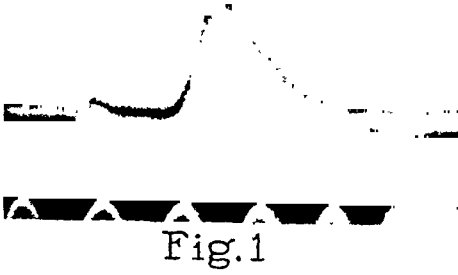
The non-rhythmic group is made up of nerves in a state approximating more closely to normal, although it may fall short of giving a faithful representation of normality. Spontaneous discharge is minimal or absent. At the end of the positive trough the level of negativity is so small that one would doubt its presence, were it not that extrapolation backward from a tetanus to a single response, as the limiting case, gives support to the interpretation that the appearance of negativity is genuine (fig. 5).

After-potentials following a tetanus. After a tetanus, the negative and positive after-potentials are accentuated, particularly the latter (fig. 5). Owing to the increased tendency toward positivity, which develops during a tetanus (Gasser, 1935), the duration of the negative after-potential following the last spike in the train is curtailed, and the positive notch which succeeds it is deepened. After the positive notch, however, a very definite period of negative after-potential may appear. The latter in turn is succeeded by a second positive period.

Further development of the second appearance of negative and positive after-potential may be achieved by increasing the duration and frequency of the conditioning tetani. A concrete example will give an idea of what happens.

A nerve was tetanized for 2.2 sec. at 300 per sec., a frequency which the nerve was able to carry with full-sized spike production. At the end of the tetanus the potential dropped to $+146\mu v$; 38 msec. after the end of the tetanus, the potential again crossed zero and reached a negative maximum of $38\mu v$ at 75 msec.; after 425 msec. it again became positive, and remained so to the end of the record which was 3 sec. later. A result qualitatively similar, but following a shorter tetanus, is shown in figure 6 a.

Freshly isolated nerves, which show rhythmicity in the after-potentials in a single response, also show it in the after-potentials following a tetanus. Corresponding with the post-tetanic augmentation of the after-potentials, the oscillations become more definite; and it also becomes clear that the base line about which the oscillation is taking place is not the line of zero potential, but one determined by the trend of the potential resulting from



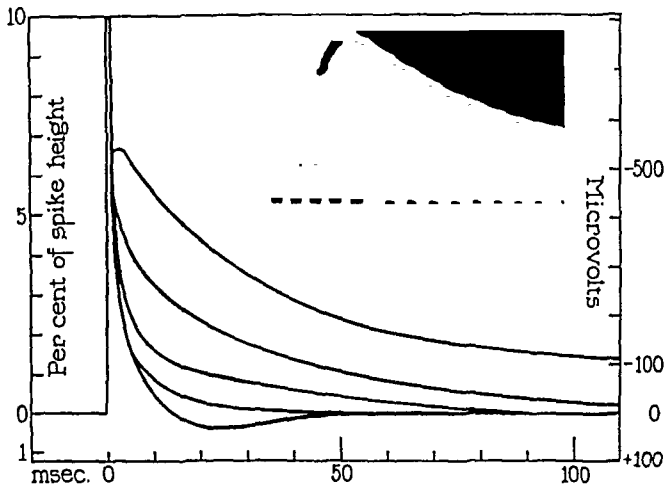


Fig. 3. Successive stages in the development of the negative after-potential in a veratrinized phrenic nerve. The first 4 curves are taken 0, 10, 18, and 28 minutes respectively after application of the drug. The top curve was taken after the nerve had been tetanized. Inset. Record of a single action-potential of a veratrinized phrenic nerve, set up soon after a tetanus. The spike is not visible. Time: 60 cycles.

the tetanus. The first oscillation is sharply negative (fig. 6 b); the second, however, comes near the transition to the positive potential, and while the oscillation is clean-cut it may attain little negativity even at its crest, and

Fig. 1. Form of mammalian spike recorded from a single fiber in a lumbar dorsal root of the cat. The spike is preceded by a shock artifact. Time: 0.2 msec. Temperature: 37.5°C.

Fig. 2. Calibration curve of D. C. amplifier. Time: 1 msec.

Fig. 4. After-potentials in a single action of a fresh phrenic nerve. The action starts at the break in the line and becomes visible in the record during the first negative after-potential. The small irregularities in the line are caused by spontaneous discharges. Time: 40 msec.

Fig. 5. After-potentials of a phrenic nerve of a cat rendered monophasic with cocaine. a, single response. The record starts at the junction of the spike and negative after-potential. If there had been a diphasic artifact, it would have appeared as a very sharp incisure at the position indicated by the arrow. b, c, tetani at two frequencies. Time: 20 msec.

Fig. 6. After-potential forms seen following a tetanus. The records show in succession the resting potential, the tetanus (with the bottoms of the curtailed negative after-potentials visible in parts b and d), and the sequence of events at the end of the tetanus. a, 0.25 sec. tetanus at 500 per sec. The first positive potential lasts 57 msec. and reaches 400 μ volts. The succeeding negativity lasts 200 msec. and reaches 90 μ volts. The second positive potential maximum is 47 μ v.; time: 0.2 sec. b, oscillating after-potential, time: 0.2 sec.; c, time: 0.2 sec.; d, time: 1 sec.

Fig. 7. Negative and positive after-potentials in a single response of a veratrinized phrenic nerve. Time: 1 sec.

Fig. 8. Tetanus of a veratrinized nerve. During the tetanus the heavy line is produced by the staircasing of the negative after-potentials. The vertical lines below the heavy line are diphasic artifacts. Time: 60 cycles.

the whole period of the oscillation may take place on the positive side of zero.

In order to obtain information about how long the terminal positive phase lasts in the action-potentials of tetanized isolated nerves, a few experiments were performed with the aid of a string galvanometer coupled with a one-stage D. C. amplifier. The amplifier which was very free from drift was built by Doctor Toennies and will be described in another connection. The nerves were mounted in the usual way and examined first with the oscillograph, in order to ascertain the proper strength of stimulation and to prove that the nerves were responding in a normal manner. The connections were then transferred to the galvanometer.

Because of its gradual ending, a definite figure cannot be given for the duration of the positive potential. No matter how long a period of time was allowed between readings, there was always some drift of the resting potential (amplifier drift was not a limiting factor); and when the nerves were fresh and the after-potentials at their best for study, sudden shifts of the string, probably connected with spontaneous firing in the nerve fibers, entered into the experiments as added complications. The durations given are times, previous to the final mergence of the potential with the changes which obscure it, at which positivity is still definitely discernible.

As is true in frog nerve, the degree of positivity does not increase in proportion to the severity of the tetanus; the positivity soon approaches a maximum, after which a further increase in tetanization expresses itself, not by an augmentation of the positivity, but by an increase of its duration. Following a 10 sec. tetanus, a representative figure for the positivity at its maximum would be 0.6–0.7 mv. This potential falls off gradually, reaching half relaxation within 15 to 30 sec. and approaching the end of visibility only after one to two minutes. After a 30 sec. tetanus, half relaxation requires more than one minute and extinction more than four minutes.

Variations in the magnitude of the parts of the sequence of potential changes occur, depending upon the preparation and the frequency of the conditioning tetanus. They rest in the last analysis on the ratio of negative to positive potential. When the negative component is small, the potential following the positive notch does not quite reach zero and a maximum occurs while the potential is still positive (fig. 6 c). Following the maximum, the positivity again increases slightly, then slowly falls off to zero, forming a section of the cycle comparable to the second positive period which results when real negativity intervenes (fig. 6 a). In this form of the cycle the after-potentials follow a sequence resembling one which has been seen in some frog nerves (Gasser, 1935, fig. 15). The positivity appears in the two parts which in frog nerve were labelled arbitrarily P_1 and P_2 (first and second positive components).

When the negative after-potential is large, the first positive wave is

carried up on the negative after-potential, so that the bottom of its trough appears on the negative side of zero (fig. 6 d). Between the two extremes all gradations occur. Among the numerous possible variations it may be assumed, on the basis of observations of the irritability of nerves *in situ*, that those in which the action-potential does not become negative for the second time, most closely resemble what happens when the nerve is in its normal physiological state.

The variations are important because of the light that they shed upon the composition of the action-potential. The first positive wave is the homologue of the positive wave as it regularly appears in a single response. A tetanus accentuates it. At the same time it causes an increased production of negative potential. According to the size and duration of the latter, the position of the positive trough is determined and also the amount of negativity residual when the opposition of the first positive wave is removed. Otherwise stated, the first positive potential is the positive potential of the single action—augmented—and intercurrent in a negative potential also augmented by the tetanus. The second positive potential must be connected with the tetanus through the increased negative potential. An increased negative potential is regularly followed by an increased positive potential and must be complemented by it.

The effect of an increased negative after-potential in a condition in which there is no simultaneous increase of the first positive potential is seen in single responses of veratrinized nerves (figs. 3 and 7). As the negative after-potential develops, it fills in the positive trough on the early side and creates new positivity beyond it; and this process goes on until the negativity lasts 30 sec. and the succeeding positivity 100 sec. The latter figures are taken from experiments with the string galvanometer in which the size of the negative after-potential attained was 1.6 mv.

Comparison of the potentials after a tetanus with the augmented potentials following a single response of veratrinized nerve reveals an analogy and a contrast. In both there is an enlarged negative after-potential followed by a positive potential; but after a tetanus there is an additional variable—an augmented positive component cutting into the negative after-potential.

When a veratrinized nerve is tetanized, the negative after-potential is so dominant that all signs of an augmented first positive potential are obliterated. Staircasing of the negative after-potential occurs to a marked degree during the tetanus; at the end of the tetanus the after-potential starts with the high negative value which has been reached and declines to the analogue of the second positive period, without sign of a first one (fig. 8).

EXCITABILITY IN RELATION TO THE ACTION-POTENTIAL. The measurement of excitability means the determination of thresholds from the begin-

ning of the action to the end of the last trace of potential change. Negative after-potential is associated with supernormal excitability (Gasser and Erlanger, 1930), positive after-potential with subnormal excitability (Graham and Gasser, 1934; Gasser, 1935). Therefore, if the rule be generally applicable, the curve of excitability should be as characteristic of the nerve and its various states as is the action-potential. We shall see that the rule holds.

Refractory period. The end of absolute refractoriness should come with the ending of the spike (Adrian, 1921), that is, very soon after 0.4 msec. In the best preparations this duration is indeed approached; refractory periods lasting between 0.41 and 0.44 msec. have been obtained in both roots and peripheral nerve. But not infrequently, no sign of a second response can be evoked unless the shock interval is 0.5 msec. The earliest responses are small and slow of conduction, but even a slight lengthening

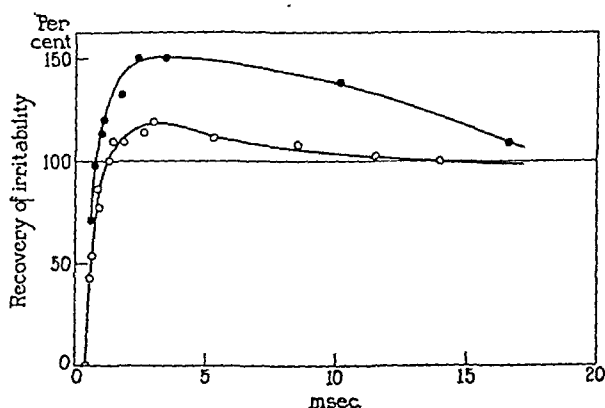


Fig. 9. Recovery of excitability in an isolated saphenous nerve (circles) and a phrenic nerve (dots).

of the interval brings out a much larger size. At the same time, the threshold falls, and at intervals as small as 1.0 msec. supernormality may already be reached (fig. 9). We shall see later that recovery of isolated nerves takes place more rapidly than recovery of nerves in the body.

From the shortness of the refractory period the inference may be drawn that a nerve can produce 2000 spikes or more per second. Upon testing the inference it is found that a rate of this kind is possible, but for short periods only, and when the stimuli are decidedly supermaximal and the lead is close to the stimulating cathode. Figure 10 shows 2000 spikes per second (upper line), combined with shock artifacts of the magnitude shown in the lower line. Some fibers are alternating, but the spike areas produced cannot be accounted for otherwise than by assuming that some of the fibers are responding to every stimulus. Records made at a distance from the cathode, when the frequency of stimulation is 2000 per second are not susceptible of a detailed interpretation, because of the confusion of tem-

porally dispersed spikes and shock artifacts. One point, however, is clear. The amount of alternation occurring is much greater at a distance than near the cathode, showing that many of the impulses set up by the strong shocks encounter some point in the nerve which they cannot pass.

At 1 msec. recovery has so far advanced that a second response has a large fraction of normal size, the fraction depending upon the refractory periods of all the fibers of the nerve and upon the state of the nerve. For example, even the threshold fibers of the two nerves presented in figure 9 would not produce maximal spikes at the same intervals. None of our preparations has ever shown complete recovery of size in 1 msec. Eighty per cent recovery, as seen in figure 11, is a fair example. Spikes cannot be maintained at this level, however, when they follow one another at millisecond intervals. Even in the first line of figure 11 some falling off is apparent, and the decrease progresses continuously until some fibers begin to drop out and alternation sets in (third line).

Supernormal period. Following the relatively refractory period, the excitability of isolated nerves becomes supernormal. In many preparations the excitability rises to between 120 and 150 per cent of normal (fig. 9), occasionally to 200 per cent, and the supernormality lasts as long as the negative after-potential, i.e., 12 to 20 msec.

A striking method of demonstrating the supernormality during the negative after-potential is to interpolate a maximal response in the course of a series of stimuli at threshold. A description of the method follows. Through one pair of electrodes shocks are applied to the nerve from a thyatron stimulator at a rate of more than 100 per second, so that the individual shocks will not be spaced by an interval longer than the supernormal period. The strength of stimulus selected is just above threshold; a few fibers are stimulated, but for most of the fibers of the nerve the strength is subminimal. Through a second pair of electrodes, placed at a greater distance from the leads, a single maximal shock is applied. When the impulses initiated by a single shock are conducted past the pair of electrodes, through which the shocks from the thyatron are being applied, one of the latter will fall within the period of supernormality of the action and then, instead of being able to produce a response only in threshold fibers, will be able to stimulate all the fibers of the nerve. The next thyatron shock in turn will also fall in a supernormal phase, and thus the cycle is repeated (fig. 12).

A train of maximal responses cannot be set up, however, without further consequences. At the beginning of the train the negative after-potential increases, giving the appearance of staircasing in both the spikes and after-potentials (Gasser, 1935). Later, along with the tendency to produce positive after-potential, the threshold rises and fibers begin to drop out in the order of their thresholds. Once a fiber has dropped out it cannot

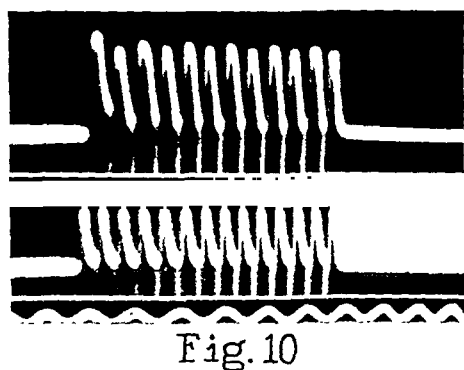


Fig. 10

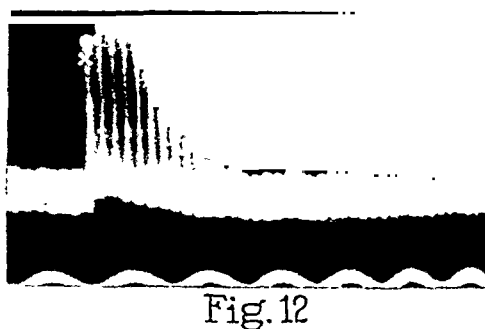


Fig. 12

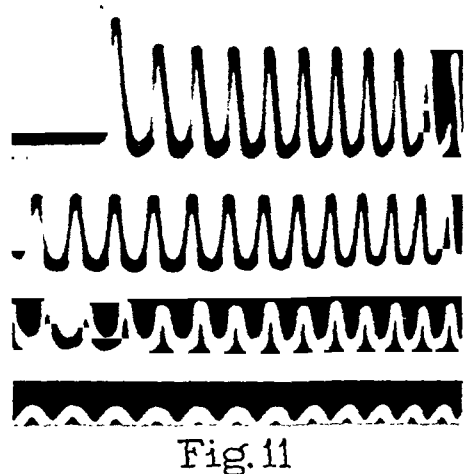


Fig. 11

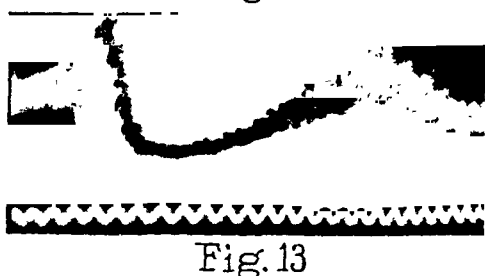


Fig. 13



Fig. 14

Fig. 10. Upper line: Short tetanus of a dorsal root at 2000 per second. Lower line: Shock artifacts recorded during reversible ether narcosis (at a slightly higher frequency). Time: 1 msec.

Fig. 11. Start and progression of a tetanus in a phrenic nerve. Frequency of stimulations: 1000 per sec. The third line follows the first by about 20 sec. Time: 1 msec.

Fig. 12. Effect on a threshold tetanus of an interpolated maximal shock, phrenic nerve. Tetanus at 325 per sec. Interpolated shock at x . Time: 20 msec.

Fig. 13. Effect of the negative and positive phases of the after-potential in a phrenic nerve upon spontaneous discharge. A single conditioning action starts at the break in the line. Time: 10 msec.

Fig. 14. Excitability in relation to the depth of the positive after-potential in the phrenic nerve. Varying depths of positive after-potential are produced by tetani of different lengths and frequencies. Testing shock applied through another electrode and at the same strength in each case. a, testing response in isolation; b, testing response after single conditioning action; c, d, e, after tetani. The added horizontal line is at zero potential. Vertical projections below the line are diphasic artifacts.

reënter, because at the time of arrival of the next shock, the irritability will have changed to subnormal. Thus the response of the nerve as a whole to the thyatron stimuli gradually returns to the condition which obtained before the interpolated shock occurred.

Subnormal period. After the supernormal phase of a single response there develops a period of subnormality, which ordinarily lasts as long as any degree of difference from the normal threshold can be detected. In the case of rhythmic nerves, however, the situation is different. While the entire course of the excitability in these nerves has not been plotted by the method of thresholds, enough points have been located to prove that the supernormality reappears with the start of the second negative potential, and disappears again with its subsidence. Successive conditioning actions, when rhythmic in character, show enough minor differences to make the application of the tedious method of plotting recovery by thresholds of doubtful value; however, a qualitative indication of the course of the excitability can be derived from the behavior of nerves discharging spontaneously. The killed end (Adrian, 1930) serves as a continuous stimulus, and the amount of the discharge follows the irritability of the nerve. During the first negative after-potential the spontaneous discharge is greatly augmented. As the negativity declines and the potential enters into the first positive portion, the discharge decreases, and it may be extinguished when the positivity reaches maximum (fig. 13). As the potential rises to a second negative maximum, the discharge increases again, and at the crest it is once more greater than in the undisturbed, steady state. The same cycle is repeated in an attenuated form in the approach from the second maximum to the third and fourth.

During the prolonged and augmented potentials following a tetanus, the relation of potential to excitability still holds; but for want of sufficient data no precise statement can be made as to how the excitability changes with the potential. That it does change may be shown by determining the subnormality at the maximum of the first positive trough. As the latter deepens at the end of tetani of increasing lengths or frequencies, the subnormality also increases (fig. 14, a, b, c, d). At the end of tetani of such extent that the absolute level of the maximum begins to rise again, because of the magnitude of the negative after-potential in which the first positive potential is an incisure, the subnormality begins to decrease (fig. 14 e). At the end of the incisure, subnormality gives way to marked supernormality.

In order to test the excitability during the remainder of the after-potential following a tetanus, a very simple procedure was followed. The amplifier was set to give about 30 mm. per mv. and a shock, thyatron-controlled as to strength, was selected to give a spike of about one-fifth of the maximal when the nerve was equilibrated to one response per two seconds. Such spikes were good indicators of changes in irritability. If

the threshold fell, more fibers were stimulated and the spikes became higher; if the threshold rose, fewer fibers were stimulated and the spikes became lower. In a series of equilibrated responses a short maximal tetanus (starting from electrodes placed on another portion of the nerve) was interpolated, and the sizes of the responses were photographed during successive sweeps of the oscillograph before and after the tetanus.

The results obtained in a sample experiment are plotted in figure 15. A very striking increase in the size of the responses occurs during the negative after-potential, and this is followed by a decrease during the positive after-potential. Thus the complete cycle through which the excitability of a fiber in such a nerve passes before returning to its steady state is made up of: the refractory period, a first supernormal period, a first subnormal period, a second supernormal period, and a second subnormal period.

In the form just described, the relation of excitability to the after-potentials is clearly defined: supernormality and subnormality in relation to negative and positive potentials; but the form is an exaggerated one, resulting from the condition of nerves in isolation. The form depends upon

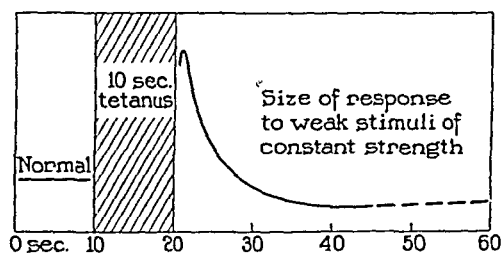


Fig. 15

both the degree of previous activity and the length of time the nerve has been mounted. Fresh nerves show less negativity and less supernormality.

Through the exaggerated form, the way is opened to an understanding of the normal form. The same processes are operative when a nerve is functioning in its proper position in the body and their presence is recognizable by the characteristic shape which they impart to excitability curves. Although the processes operate to a different extent and their effects sum up to give a quantitatively different picture, the individual features of the curve are identifiable with similar curves seen in isolated nerve. For example, if the recovery of excitability, instead of following a smooth curve, shows an early acceleration of recovery which is not maintained and is succeeded by a recession, it may be inferred, even if no actual supernormality occur, that a process making for supernormality is playing its part.

Excitability of nerve in situ. In order to gain information about the extent to which the various potentialities of a nerve are exercised when it is functioning as it does physiologically, an examination was made of nerves

in the body. After-potentials cannot be measured on a nerve *in situ* without exposing the nerve to an extent which precludes all assurance of its being in a physiological state. On the other hand, thresholds may be measured without exposure of the nerve; and through the associations existing between thresholds and potential, the nature of the latter may be inferred.

Thresholds were measured on the saphenous nerve of the cat in intact animals under general anesthesia, or in decerebrate or chronic spinal preparations. An incision was made through the skin in the upper part of the thigh over the saphenous nerve, without making a further dissection. The nerve was, therefore, unexposed and left with its natural circulation intact. Two pairs of chloride-covered silver-wire stimulating electrodes were placed in a vertical position over the surface of the thigh, in a way so that their tips came in contact with the fascia immediately overlying the nerve. The conditioning stimuli were supplied through the proximal electrodes, and the test shocks through the distal electrodes. In this way, local effects were avoided. Precaution was taken to prevent cooling of the limb. In many preparations the nerve was blocked intra-abdominally by a ligature, in order to prevent interference by reflex movements.

The effect of testing shocks was recorded from a portion of the nerve exposed below the knee. The nerve was drawn through a glass tube, into the sides of which silver wires had been sealed in order to make connection with the amplifier and oscillograph. When the tube was fastened in place, it served as a very effective moist chamber, the nerve staying in good condition for hours. It was, of course, not essential to the experiment that this part of the nerve be in a completely physiological condition, as it served merely as an indicator of whether or not a response had been started by the testing stimulus applied to the protected central portion of the nerve. Throughout all the measurements the testing shocks were adjusted to a strength which would produce a response of constant height, the amplification being such that the height selected involved a small group of the lowest threshold fibers of the nerve. Excitability was thereby defined as the reciprocal of the shock strength which would excite a constant number of fibers. As variation in the recovery curves would be expected chiefly in the portions connected with supernormality and subnormality, the course of the recovery was not plotted earlier than the later part of the relatively refractory period. Thereby the nerve was spared exposure to the strong shocks necessary for measuring early recovery.

A few early experiments were performed under general anesthesia with ether or dial, but in order to avoid possible effects of the narcotics, most of the experiments were carried out on decerebrate preparations, from which the ether administered during the decerebration had been completely eliminated. The nerve was then found to remain for six hours or longer in a steady state suitable for experimentation.

As the activity of nerve is known to be susceptible to changes in the acidity of the medium about it, and because of the possibility of the production of an acidosis in the process of narcotization and decerebration, a short, final series was carried out on chronic spinal cats. Samples of blood were taken from the latter for determination of the pH; the values found ranged between pH 7.3 and pH 7.4.¹ As between the two reactions, no significant difference could be made out in the excitability curves. Whatever change was entailed by a reaction of pH 7.3, in place of the normal pH 7.4, was obliterated by the random variations. Nor was there any significant difference between this series and the one carried out on decerebrate animals; consequently we believe that the curves as described fall within the limits of what may properly be called normal nerve functioning.

In the body, as outside, the excitability curve is characterized by a rapid rise to a maximum, a decrease from the maximum, and a long subnormal period. In nearly all the experiments, in which the conditioning excitation was a single shock, the maximum was at a definitely supernormal level (in 27 of 30 decerebrate preparations, in 4 of 5 with dial, in 2 of 3 with ether, and in all the chronic spinal preparations). Relative refractoriness ended and supernormality began 2.5 to 5.0 msec. after the start of the action. Maximum supernormality was reached after 5 to 10 msec., and the transition from supernormality to subnormality occurred at 12 to 18 msec. Subnormality was greatest at 25 to 35 msec., and there was a return to normal at 60 to 80 msec. (These durations are the ones which guided the designation in an earlier section of the paper of what must be the normal duration of the after-potentials.) The absolute degrees of supernormality and subnormality varied somewhat from animal to animal. Supernormality varied from 0 to 20 per cent at the peak, with an average of 7 per cent. Subnormality varied from a just detectable amount to 6 per cent, with an average of 3 per cent.

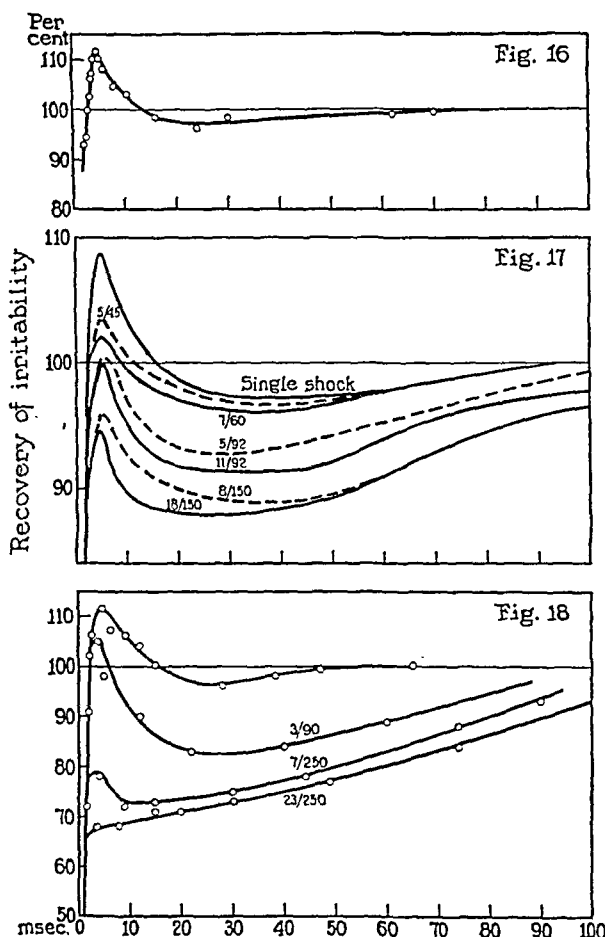
Figure 16, taken from one of the chronic spinal preparations, shows a typical curve. Compared with the curves obtained from isolated nerves, the principal difference is found in the supernormal phase, which is less in degree and shorter in duration. The relatively refractory period is longer. In isolated nerve the relatively refractory period is shortened by the augmented negative after-potential process, in accordance with a mechanism analogous to that described by H. T. Graham (1934) for frog nerve.

When the number of shocks in the conditioning excitation was increased, the same general form of excitability curve was found, but at all intervals between the conditioning excitation and the testing shock, the excitability

¹ We are indebted to Dr. J. E. Lehmann for the making of the measurements and to Dr. L. Michaelis for the use of his glass electrode and accessory apparatus.

was decreased, and in relation to the degree of decrease the period of subnormality was extended.

Figure 17 presents a family of curves in which a comparison is made on the same nerve of the course of recovery following a single conditioning shock, with that resulting from conditioning tetani of various duration and



Figs. 16, 17, 18. Recovery of the saphenous nerve of the cat *in situ*. Figure 16, chronic spinal cat. Single conditioning shock. Figures 17 and 18, observations on two nerves in decerebrate animals. Conditioning action changed in the course of the experiment from a single shock to tetani. The number of shocks used and their frequency are indicated; e.g., 5/45 means 5 shocks at 45 per sec. Figure 18, upper curve conditioned by a single shock.

frequency. A few shocks in the conditioning tetanus were sufficient to bring the initial maximum below the level of 100 per cent excitability, and a greater number carried it still lower. High frequency of shocks was more effective in this respect than a large number of shocks. At the same time, the decline in excitability following the maximum was more rapid, the

maximum of subnormality came earlier, and the area subtended by the initial peak rise was decreased. When a still more intense conditioning stimulation was applied, the peak disappeared altogether, all that was left being a shoulder on the curve of recovery. Figure 18, taken from another nerve, shows the course during a development of this kind. Here a subnormality amounting to 30 per cent was produced. The after-potential which would correspond to the lower curves in this figure would in all probability resemble that pictured in figure 6 c.

Long-lasting subnormality follows severe tetanization. In one experiment a tetanus for 15 sec., at the rate of 300 per sec. produced subnormality for 20 sec., and a similar 30 sec. tetanus, subnormality lasting 30 sec. In another experiment, subnormality of one minute succeeded a tetanus lasting one minute.

In the description of the experiments on isolated nerve the prolongation of the positive after-potential by tetanization was pointed out. Despite the aid to be derived from an active circulation, a similar process must occur in the body; otherwise the subnormality could not be accounted for. The amount of negative after-potential, on the other hand, must be very much less than in isolated nerve. Augmentation of the latter to the point of introducing an intercalated supernormal phase, as happens in isolated nerve, must be considered as being a distinctly unphysiological distortion. A search for a second period of supernormality in the body has thus far led to negative results only; but whether or not there are any signs of an analogous effect can only be decided after the mapping of recovery following a severe tetanization has been given a more careful study than any heretofore attempted.

DISCUSSION. One of the most important questions in connection with this investigation has been: Is the supernormal phase physiological? On the basis of our experience, we feel that it is physiological. While our experiments were in progress, observations made on the recovery curves of rabbit oculomotor and sciatic nerves *in situ* were published by Lorente de N6 and H. T. Graham (1935). Our findings are completely in agreement with theirs. They found, however, that in rapidly made preparations, in which readings could be started within an hour after the beginning of the experiment, supernormality was absent, although it appeared later; and they raised the question whether the true normal state is not better represented in these early observations than in the later ones.

We have not examined nerves during the first hour after decerebration. Our observations were all started later than one hour, and they extended over a period of many hours. During this long period the nerve remained in a state which was satisfactorily constant, and we had every reason to believe that this period—rather than an early one, in which possible effects of residual anesthetic or abnormal breathing arising from the manipulation

might have operated—offered the most favorable time for the making of readings. Confirmation of the results so obtained by those derived from chronic spinal cats supported that opinion. Also, no difference was found between curves obtained soon after application of the electrodes and those obtained hours later. Consequently we feel that the nerves used were well within the limits in which nerves must function during ordinary body activity.

While the existence in the body of a process making for supernormality may be considered as being established, absolute supernormality cannot be of great importance to a nerve in its function of carrying messages, as it is seen only when the excitability after a single response, or a very few responses, is compared with that of a quiescent nerve; that is, under conditions which differ from the ordinary mode of occupation of nerve fibers by actions. Nerve messages are carried by a series of actions, and following a series absolute supernormality is not seen in nerves *in situ*. The importance resides rather in the process which produces supernormality. *The recovery of excitability must be greatly hastened by it.* Instead of following an ordinary exponential curve, the course of recovery shoots far ahead of a curve of this kind and reaches an early maximum, although it does not achieve absolute supernormality. In the exaggerated form in isolated nerve, the acceleration of recovery is such that excitability may rise from absolute refractoriness lasting more than 0.5 msec. to supernormality occurring earlier than 1.0 msec.

From studies made of peripheral nerve it is desirable to get a composite picture of the properties of nervous tissue; therefore, a comparison of mammalian and frog nerves is useful. The idea that there is a set of common properties is supported by the fact that all the phenomena which have been worked out on frog nerve may be recognized qualitatively in mammalian nerve: a negative after-potential varying with the conditions about a fixed spike; a relatively refractory period shortened by development of the negative after-potential process; supernormality varying with the amount and duration of negative after-potential; a positive after-potential augmented by tetanization or by conditions which increase the negative after-potential; and subnormality associated with the positivity.

The points which are shown more clearly by mammalian nerve than by frog nerve are: positivity after a single response; the division of the positive after-potential into two parts; and the concurrence of processes which, on the one hand, tend to produce negativity, and on the other hand, positivity.

On the theoretical side the impression has gained support that the first positive potential is associated with spike production, and that the second positive potential is secondary to the negative after-potential process. A simple sequence of negative and positive after-potentials is seen in single responses of veratrinized nerves. The same sequence at the end of a

tetanus of unpoisoned nerve is complicated by the augmented first positive potential cutting into the negativity.

SUMMARY

The properties of the action-potential of mammalian A fibers are described.

Measurements of the duration of the spike in single fibers of spinal roots show that the major portion of the spike is completed in 0.4 to 0.43 msec.

The spike is succeeded by processes giving negative and positive potential signs. These potentials sum algebraically and the absolute value of the potential at any time depends upon the individual momentary values of the potentials. Immediately following the spike, the after-potential is negative. The negative after-potential varies independently of the spike, and in veratrinized nerve it may be shown to have a rising phase. Succeeding the negative period, a positive after-potential is always present in normal fibers. On the average its magnitude amounts to 0.2 per cent of the spike height.

In freshly mounted isolated nerves, in which the fibers are subject to spontaneous discharges, the after-potential is rhythmic.

Tetanization increases both the negative and the positive after-potentials. The positive potential then appears in two parts. The first part is analogous to that following a single spike; it has the same duration as the latter, but is larger. The second part has a duration as well as a size which is related to the severity of the tetanus. After a maximal tetanus of 30 sec. the potential is $+0.6$ to 0.7 mv., and the duration more than four minutes. The increased negative after-potential may cause the two parts of the positive potential to be separated by a period of absolute negativity, or in other instances by a negative crest still on the positive side of zero. The variations are described.

The augmented negative after-potential of veratrinized nerve is succeeded by a large positive potential which seems to be analogous to the second part of the positive potential following a tetanus.

The refractory period lasts 0.41 to 0.44 msec. in the best preparations. Tetani at 1000 and 2000 per second are described.

Recovery of excitability was studied in nerves functioning under physiological conditions in decerebrate and chronic spinal cats. A typical set of excitabilities in a single response may be described as follows: relatively refractory period, 3 msec.; supernormality between 3 and 15 msec., with a maximum of 7 per cent at 7 msec.; subnormality between 15 and 70 msec., with a maximum of 3 per cent at 30 msec. The durations of the supernormality and the subnormality define the normal durations of the negativity and positivity of the after-potential.

Following a tetanus, the excitability as shown in all parts of the curve is

depressed, but except after very severe tetani, the typical form of the curve is preserved: a rapid rise, a secondary fall, and a slow recovery. The total duration is increased in relation to the severity of the tetanus. A one minute tetanus is followed by subnormality of one minute.

In isolated nerves in oxygen there are quantitative differences from the condition obtaining in the body. The relatively refractory period is shorter and the supernormality greater, because of an abnormally large negative after-potential. Following a tetanus, the excitability depends upon the course of the after-potentials. In cases where the negative after-potential is sufficiently augmented along with the positive after-potential, the course is: relatively refractory period, first supernormal period, first subnormal period, second supernormal period, and second subnormal period.

REFERENCES

- ADRIAN, E. D. *J. Physiol.* **55**: 193, 1921.
Proc. Roy. Soc. B. **106**: 596, 1930.
BLAIR, E. A. AND J. ERLANGER. *This Journal* **106**: 524, 1933.
GASSER, H. S. AND J. ERLANGER. *This Journal* **94**: 247, 1930.
GASSER, H. S. AND H. T. GRAHAM. *This Journal* **101**: 316, 1932.
GASSER, H. S. *This Journal* **111**: 35, 1935.
GRAHAM, H. T. *This Journal* **110**: 225, 1934-1935.
GRAHAM, H. T. AND H. S. GASSER. *Proc. Soc. Exper. Biol. and Med.* **32**: 553, 1934-1935.
LORENTE DE NÓ, R. AND H. T. GRAHAM. *Proc. Soc. Exper. Biol. and Med.* **33**: 512, 1935-1936.

THE UTILIZATION OF FRUCTOSE IN THE MAMMALIAN ORGANISM AS SHOWN BY EXPERIMENTS ON HEPATOMIZED AND EVISCERATED PREPARATIONS¹

JEAN P. GRIFFITHS AND E. T. WATERS

From the Department of Physiology, University of Toronto

Received for publication May 18, 1936

The preponderant weight of opinion has been in favor of the view that carbohydrate foods are first converted to glucose before oxidation in mammals, and much attention has been focussed therefore upon the mechanism and site of such conversion. The extent to which other sugars can substitute glucose has been regarded as depending upon the rate at which they were converted to glucose. On the other hand, it is still a moot point as to whether sugars other than glucose are always converted to glucose before they can be oxidized by the different tissues of the mammalian organism. The experiments recorded in this paper were undertaken in the hope of elucidating these problems in the instance of the sugar fructose, a common constituent of our diet, chiefly in the form of cane sugar.

Carbohydrate foodstuffs are absorbed into the portal system from the intestine in the form of monosaccharides. What chemical changes, if any, take place during passage through the wall of the intestine have not been definitely established. While it has been conclusively shown that fructose can be absorbed as such, that a certain amount of conversion to glucose may and does take place during absorption is a fact not yet demonstrated.

That the liver possesses the ability to convert fructose to glucose has been conclusively shown by a number of workers in *in vitro* and *in vivo* experiments. Indeed this well-known ability of the organ to effect this conversion has been used extensively as an efficiency test of hepatic function under varying conditions. But in passing it should be mentioned that the work of Mann and Bollman (1926) demonstrates the extreme unreliability of this test.

Bollman and Mann (1931) showed that the liverless dog could be maintained in as good physiological condition for a considerable period by an infusion of fructose as by that of glucose. These authors also found an increase in blood glucose when fructose was injected intravenously into the

¹ A report of this work was given at the Annual Meeting of the Royal Society of Canada, May, 1935.

liverless dog. This finding they have interpreted as indicating that fructose is first converted into glucose, and the glucose so formed then oxidized in these animals. Further, the fact that they were unable to show any utilization of fructose in their eviscerated dogs suggested to them that the mucosal cells of the intestine are responsible for such conversion in the liverless animal.

EXPERIMENTAL PROCEDURES: *Chemical.* True blood sugar values of our animals were obtained on Somogyi zinc hydroxide filtrates using the modified copper reagent of Shaffer and Somogyi (1933). In the absence of fructose the figures indicated the true glucose content of the blood. For the estimation of fructose we have employed two methods, that of Harding and Nicholson (1933), and the method of Campbell and Hanna (1926). The former is a fermentative method in which use is made of the selective action of a certain strain of *proteus vulgaris*. This organism quantitatively removes glucose from dilute solution, while fructose is unattacked when present in low concentration. The stock culture² of this organism has been maintained in nutrient broth and when required planted out on glycerine-agar. These departures from the method as originally given lead to considerable simplification of this part of the work and in no way diminish the yield of growth nor its fermentative properties. Given adequate facilities for the culturing in bulk of the *proteus vulgaris* we regard the method as eminently feasible. Even so, whenever this method was employed, we have always conducted control tests on the fermentative powers of the carefully washed organisms, their ability to remove completely from solution an amount of glucose greater than that of any of the blood filtrates under test, and further their inability to remove any fructose. In the original method tungstic acid filtrates of blood were used. We have employed this organism to remove glucose from zinc hydroxide filtrates. The small amount of zinc which remains in the filtrate does not affect the glucose-removal power of the organism. After centrifugation of the filtrates following incubation with the *proteus vulgaris*, the opalescent supernatant can be completely cleared by shaking with a little Lloyd's reagent and filtering through fluted filter paper.

A chart for use with the Campbell and Hanna permanganate titration method of fructose estimation was constructed based on recovery figures of fructose when added to tungstic acid blood filtrates.

Operative. The hepatectomy operations were carried out on dogs

² We are greatly indebted to Doctor Nicholson, of the Department of Pathological Chemistry of this University, for supplying us with the initial strain of *proteus vulgaris*, and for help during our preliminary trials of the method. Our thanks are also due to Dr. D. T. Fraser, Department of Hygiene and Preventive Medicine, for helpful suggestions relating to the growth, and for generous supplies of cultures of this organism throughout the course of the work.

according to the one-stage technic described by Markowitz, Yater and Burrows (1933). The method of evisceration employed was suggested to us by our colleague Dr. J. Markowitz.

The essential departure from the customary technic is the use of a pyrex glass cannula in the inferior vena cava, as in the hepatectomy operations, which is a distinct improvement over the usual practice of ligating this vein and trusting to the establishment of an adequate collateral circulation. The urinary bladder, kidneys and suprarenal glands were not removed. These animals made good recoveries.

It was found that clamping the lower end of the esophagus resulted in persistent retching movements following recovery from the anesthetic,

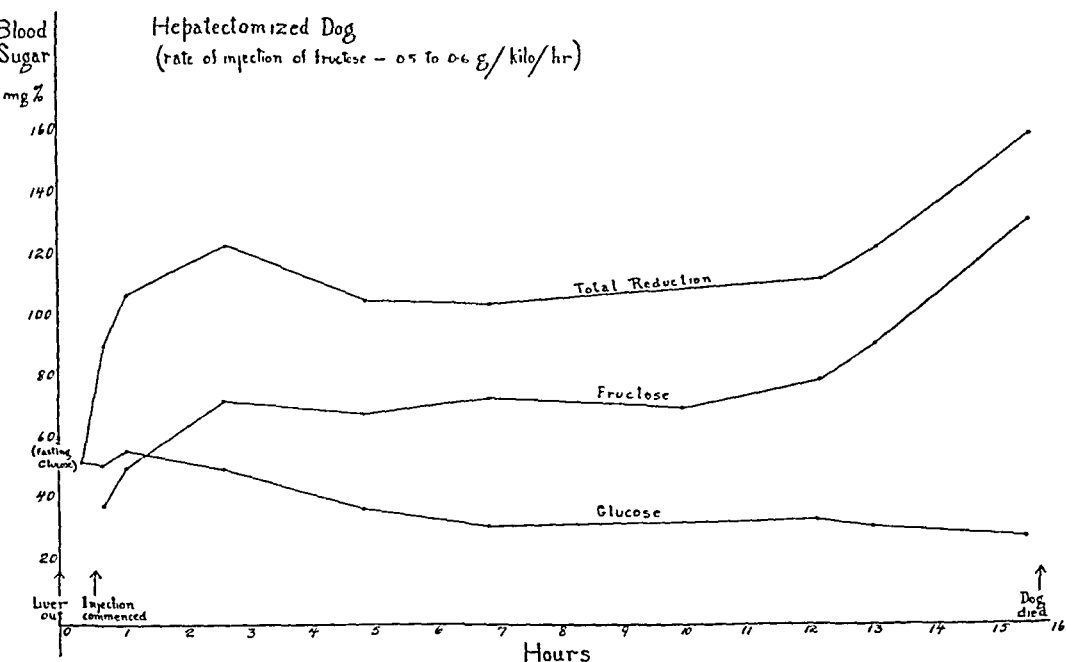


Fig. 1. ♀ Weight = 11.0 kgm. Pre-operative fast 24 hours. P.M. findings: negative. Total bloody fluid in abdomen 75 cc. (cell vol. = 11 per cent).

whereas when the clamp was placed one centimeter below on the walls of the cardiac end of the stomach these movements were considerably diminished. The troublesome ropy mucus was removed from the esophagus by washing out with several lots of water.

EXPERIMENTAL RESULTS. For the sake of brevity single experiments only are reported, but the results of these experiments have been amply confirmed in several other experiments conducted on similar preparations. The glucose-free fructose was usually administered in a 25 per cent solution containing 0.8 per cent sodium chloride, by continuous intravenous injection into the saphenous vein, using a constant injection pump.

Figure 1 shows the blood sugar levels in a hepatectomized preparation

TABLE 1

Concentrations of glucose and fructose in the blood of hepatectomized dog after intravenous injections of fructose

SAMPLES	TIME AFTER REMOVAL OF LIVER	1	2	3	4	5	6	7	8
		METHOD							
		Zinc precipitation and proteus fermentation			Tungstate precipitation and KMnO ₄ titration				
		Total reduc- tion	Fructose		Glucose 1-2	Total reduc- tion	Fructose		Glucose + non-ferment- able reduc- tion 5-6
		Calculated as mgm. per cent glucose	Calculated as mgm. per cent glucose	Mgm. per cent fructose	Mgm. per cent glucose	Calculated as mgm. per cent glucose	Calculated as mgm. per cent glucose	Mgm. per cent of fructose	Calculated as mgm. per cent glucose
1	Pre-operative	72.3			72.3	86.0			86.0
2	30 min.	81.7			81.7	99.7			99.7
3	46 min.	67.7			67.7	80.5			80.5
0.5 gm. fructose per kgm. in- jected	46 min. to 48 min.								
4	57 min.	198.0	126.0	134.0	72.0	210.0	129.8	138.0	80.2
5	1 hr. 25 min.	136.7	72.7	76.7	64.0	152.0	81.7	86.9	70.3
0.5 gm. fructose per kgm. in- jected	1 hr. 25 min. to 1 hr. 26 min.								
6	1 hr. 37 min.	218.0	156.7	166.3	61.3	238.0	166.3	177.0	71.7
7	2 hr. 5 min.	151.3	94.3	100.0	57.0	168.7	100.5	106.9	68.2

The fructose concentrations as estimated by the KMnO₄ titration (column 7) are higher than those obtained after proteus fermentation (column 3). The reason for this discrepancy is not clear although it may be accounted for in part by the rather indefinite end-point of the KMnO₄ titration. In other experiments the agreement was frequently much closer. However, by neither method was any rise in blood glucose indicated after the ingestion of fructose. It should be stressed that the difference in reduction of the copper reagent by glucose and fructose is taken into account and is shown in columns 2 and 3 and 6 and 7. To obtain the correct glucose concentration the fructose must be calculated as glucose and subtracted from the total reduction which is also calculated as glucose. Although the KMnO₄ method estimates fructose directly, each figure so obtained was converted into the equivalent glucose figure as would be given by the copper reduction method. This value could then legitimately be subtracted from the total reduction (as mgm. per cent glucose) of the blood sample to give the glucose content plus the non-fermentable reduction in the tungstate filtrate. There is no significant variation in the non-fermentable reduction values of such blood filtrates. The table will suffice to show that inattention to this difference in the copper reducing powers of glucose and fructose would lead to very appreciable errors especially in those samples containing relatively high concentrations of fructose.

receiving a continuous intravenous injection of fructose at constant rate. The concentration of fructose in the blood remains practically the same until towards the end of the experiment, when there is an increase in the fructose concentration showing a marked decrease in utilization of this sugar prior to the death of the animal. It will be seen that the concentration of blood glucose remains remarkably constant after the initial fall.

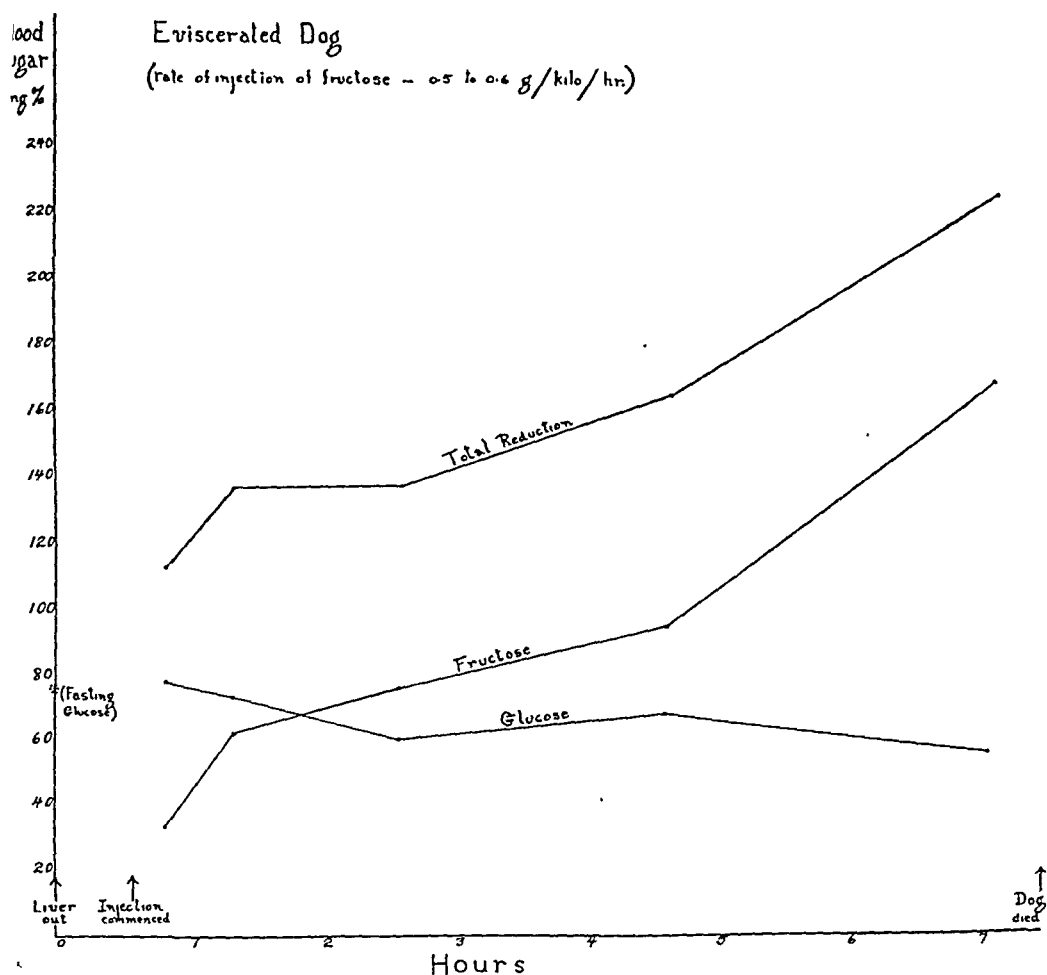


Fig. 2. ♂ Weight = 9.3 kgm. Pre-operative fast 24 hours. P.M. findings: negative.

One interpretation of this constancy of glucose concentration would be that the fructose was converted to glucose at the same rate as that of the oxidation of glucose. But the fact that no change occurred in the concentration of blood glucose on varying the rate of injection of fructose leads us to doubt the validity of this hypothesis. Thus in table 1 it will be seen that there is no appreciable change in the concentration of blood glucose, even when the fructose is injected rapidly in rather massive amounts. In

this respect our results are in definite contradiction to those of Bollman and Mann, who found a marked increase in the concentration of blood glucose in the hepatectomized animal following such an injection of fructose. These authors used the Campbell and Hanna method for estimating blood fructose. We are unable to offer any explanation for this divergence of experimental results. We have repeated this experiment several times, both methods of fructose estimation have been used, and in no case have we obtained either an increase or a decrease in glucose concentration which was outside the limits of our experimental error. We regard this experimental finding as of considerable importance in elucidating the problem as to whether conversion of fructose to glucose necessarily takes place prior to oxidation. Bollman and Mann could find no other obvious interpretation for their experimental result, and indeed it was almost entirely upon this finding that they favored the conversion theory.

Figure 2 shows the levels of the blood sugars in an eviscerated preparation receiving a continuous intravenous injection of fructose. While the glucose concentration shows little variation, the decrease in rate of utilization of fructose towards the end of the experiment is again apparent. Neither this animal nor similarly treated animals showed any sign of hypoglycemic convulsions. They lived for about $6\frac{1}{2}$ to $7\frac{1}{2}$ hours after completion of the operation. The longest survival period of control eviscerated dogs receiving no sugar or other injection was three hours. In some of these experiments, a determination of free sugar of muscle was carried out seven hours after the evisceration. No attempt was made to differentiate between glucose and fructose, but the total free sugar was in accord with expectation and at least serves to show that there was no undue accumulation of free sugar in muscle, and hence further assurance that the injected fructose was definitely being utilized by the eviscerated animal. In our eviscerated preparations the inferior vena caval blood flow was uninterrupted, whereas in Mann and Bollman's experiments the inferior vena cava was tied and the establishment of an immediate efficient collateral circulation relied upon. The difference in operative procedures may account at least in part for the evident difference in behavior of their preparations and ours following the administration of fructose.

One of the hepatectomized preparations following the belated administration of fructose, given continuously at the rate of 0.4 gram per kgm. of body weight per hour, began to show slight convulsive movements about two hours after the commencement of injection. The blood glucose concentration at the time of the onset of muscular twitches was 25.9 mgm. per cent; that of fructose 55.7 mgm. per cent. In spite of the marked increase in the rate of fructose infusion to 1.3 gram per kgm. per hour there was no material increase in the glucose concentration (27.0 mgm. per cent after $\frac{1}{2}$ hour, when fructose value was 104 mgm. per cent) and hypoglycemic

symptoms persisted. But there was no progressive increase in the severity of the movements, the symptoms remaining of a very mild type over a period of three-quarters of an hour. An injection of glucose ($\frac{1}{2}$ gram per kgm.) at this stage was promptly followed by complete disappearance of all hypoglycemic symptoms and well-marked general improvement in the condition of the animal. Although the hypoglycemia was not abolished by the fructose we feel that this sugar prevented the development of more vigorous symptoms.

DISCUSSION. In the interpretation of our experimental results we feel due consideration must be given to the fact that in both hepatectomized and eviscerated preparations receiving fructose there is no material increase in the blood glucose concentration. We should like to stress that as far as a review of the experimental data is concerned, there is nothing at variance with the hypothesis that in mammals fructose can be directly oxidized. While in some types of tissues fructose may be directly oxidized, in others this may not be possible, conversion to glucose being first necessary. This conversion may take place in such tissues or the glucose may be derived from a possible conversion of fructose to glucose by some other tissue in excess of its own carbohydrate requirements, the net result being no substantial alteration in the total quantity of free glucose as indicated by the constancy of the blood glucose concentration.

The two chief tissues concerned with the consumption of carbohydrate are the skeletal musculature and the brain. Bornstein and Völker (1929) showed that a perfused isolated mammalian limb utilized 0.25 gram fructose per kilogram as compared with 0.225 gram glucose. The muscles may first convert the fructose to glucose which is then oxidized, or the oxidation of fructose may take place directly. Regarding the energy requirements of brain, Holmes (1934) has recently stated "There is now abundant evidence that the brain as a whole has a high metabolic rate, and that its metabolic demands are chiefly for carbohydrate." Loebel (1925) first showed that brain tissue can utilize fructose without preliminary conversion to glucose, for no lactic acid appears to be formed as is the case with the metabolism of glucose in brain tissue.

Bollman and Mann were unsuccessful in their attempts to alleviate the symptoms of extreme hypoglycemia in the liverless animal by administration of fructose. We failed to abolish mild hypoglycemic convulsions with fructose, but we feel the development of more vigorous symptoms was prevented. Administration of fructose did not lead to any increase in the blood glucose, nor presumably in the glucose concentration of the tissues, and the symptoms persisted. But the *in vitro* experiments of Loebel with brain and of Gerard and Meyerhof (1927) with nerve trunks and later those of other workers show such tissues can oxidize fructose as readily as glucose. The cause of the nervous stimulation of hypoglycemia therefore

appears to be due specifically to a marked lowered glucose concentration or that of a glucose intermediary (cf. tetany due to a lowered calcium concentration) but not presumably because of a lack of readily oxidizable carbohydrate pabulum.

It might be suggested that in our animals showing hypoglycemic convulsions there may be no upset in the metabolism of the nervous system proper because, as already indicated, fructose may be an adequate substitute for glucose in such tissue, but that the initial irritation arises in some other tissue (e.g., end organs) unable to oxidize fructose and dependent upon a sufficient supply of glucose.

SUMMARY

The ability of the mammalian organism to utilize fructose in the absence of the liver has been confirmed. It has further been demonstrated that the life of the eviscerated animal can also be prolonged for a considerable time by administration of fructose, conclusively showing that in the absence of the liver and intestinal tract fructose is assimilated by an animal. In neither the hepatectomized nor the eviscerated animal was there an increase in the blood glucose following a large injection of fructose. This finding lends no support to the view that the fructose is first converted to glucose before utilization by muscle and brain and presumably by other tissues also, rather the experimental facts find a ready interpretation on the basis that in mammals fructose can be directly oxidized.

Our thanks are due to Dr. J. Markowitz for his ever ready assistance in the surgical procedures involved in this work, and to Dr. C. H. Best for similar help and for his interest in these researches.

REFERENCES

- BOLLMAN, J. L. AND F. C. MANN. *This Journal* 96: 683, 1931.
BORNSTEIN, A. AND H. VÖLKER. *Biochem. Ztschr.* 209: 103, 1929.
CAMPBELL, W. R. AND M. I. HANNA. *J. Biol. Chem.* 69: 703, 1926.
GERARD, R. W. AND O. MEYERHOF. *Biochem. Ztschr.* 191: 125, 1927.
HARDING, V. J. AND T. F. NICHOLSON. *Biochem. J.* 27: 1082, 1933.
HOLMES, E. G. *Ann. Review Biochem.* 3: 392, 1934.
LOEBEL, R. O. *Biochem. Ztschr.* 161: 219, 1925.
MANN, F. C. AND J. L. BOLLMAN. *Arch. Path. and Lab. Med.* 1: 681, 1926.
MARKOWITZ, J., W. M. YATER AND W. H. BURROWS. *J. Lab. and Clin. Med.* 18: 1271, 1933.
SHAFFER, P. A. AND M. SOMOGYI. *J. Biol. Chem.* 100: 695, 1933.

IN VITRO ACTION OF CRYSTALLINE VITAMIN B₁ ON PYRUVIC ACID METABOLISM IN TISSUES FROM POLYNEURITIC CHICKS¹

W. C. SHERMAN AND C. A. ELVEHJEM

From the Department of Agricultural Chemistry, Madison, Wisconsin

Received for publication June 8, 1936

As a result of investigations which were started in 1929 by Kinnersley and Peters, the conclusion was recently reached (1) that vitamin B₁ is indirectly concerned with the oxidative removal of lactate and pyruvate in avitaminous pigeon brain. It appeared that the vitamin catalyzed the coupled oxidation of some unknown substance in the presence of lactate or pyruvate. However, it could not be determined from their work which was immediately concerned in the interaction. In similar studies carried out in our laboratory on polyneuritic chicks, we (2) were unable to show any relationship between vitamin B₁ and lactate oxidation in brain tissue. In avitaminous heart tissue, on the other hand, an indirect relationship was indicated. By means of oxygen uptake studies it was shown that avitaminous heart had a subnormal ability to respire in lactate. Lactic acid analyses showed a decreased rate of lactate removal by avitaminous heart. Additions of vitamin B₁ had a stimulating effect upon the rate of respiration. It appeared, however, that this lowered ability of certain tissues from polyneuritic chicks to metabolize lactate was a secondary effect of the avitaminosis. It was found that additions of small amounts of pyruvate to heart and kidney exerted an inhibitory influence upon lactic acid dehydrogenase activity. This inhibition was especially marked in avitaminous tissues. It was demonstrated by Thompson and Johnson (3) that bisulphite-binding substances increase in the blood stream of pigeons and rats during polyneuritis. Recently Johnson (4) has succeeded in isolating the bisulphite-binding substance in the form of the 2:4 dinitrophenylhydrazine from the blood of B₁ deficient pigeons in sufficient quantities for characterization and has shown it to be pyruvic acid.

In the present communication results are presented from oxygen uptake studies upon tissues from normal and polyneuritic chicks using pyruvate as substrate. Additions of vitamin B₁ to avitaminous tissues respiring in pyruvate substrate were made. Results from chemical determination of

¹ Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

the pyruvate removed by normal and avitaminous tissues and by avitaminous tissues with added vitamin are given. We find that in avitaminous cerebrum and kidney there is a decreased rate of respiration in pyruvate substrate. This lowered oxygen uptake was brought back to a more nearly normal level by the addition of crystalline vitamin B₁. Measurement of pyruvate removal shows a lowered rate of removal in avitaminosis. The *in vitro* addition of vitamin B₁ increased the removal of pyruvate in avitaminous tissue.

METHOD. The techniques employed in the production of acute polyneuritis in the experimental chicks and in the subsequent removal of tissues was identical with the methods we have previously described. The amount of minced tissue introduced into the Barcroft flasks was in all cases 0.150 to 0.160 gram for cerebrum, and 0.110 to 0.120 gram for kidney. The amount of tissue was kept uniform because the relative concentration of pyruvate with respect to tissue was found to be of importance, especially when studying pyruvate removal. Ringer phosphate-pyrophosphate buffer pH 7.3, which was M/30 in phosphate (Na) and M/100 in pyrophosphate (Na), was used throughout. The pyruvic acid was obtained from the Eastman Kodak Company and was freshly distilled *in vacuo* (37–40°C.) for use as substrate after being adjusted to pH 7.3 with NaOH. Concentrations of substrate are given in all experiments. The vitamin used in these studies was Merck's crystalline vitamin B₁.

An aqueous solution of the crystals was made to contain 25 gamma per cubic centimeter, and 0.1 cc. aliquots containing 2.5 gamma were added directly to the respirometer flasks containing minced tissue, buffer, and substrate to make a total volume of 3.0 cc. In earlier studies we used a vitamin B₁ concentrate (Oryzanin Fortior "Sankyo"). We have found these two vitamin preparations to be similar both in antineuritic activity and in their *in vitro* action. For the chemical determination of pyruvate removal by chick tissues the method of Clift and Cook (5), which depends upon the ability of sodium bisulphite to form an addition compound with pyruvic acid in acid solutions, was adopted. Results from this method have been shown by Peters and Thompson (6) to be in good agreement with results obtained using a modification of the Case 2:4 dinitrophenylhydrazine method (7) when pyruvic acid is used as substrate. Although there are other bisulphite-binding substances in biological materials besides pyruvic acid, this method affords a satisfactory means of following the removal of added pyruvate by tissues.

Determination of pyruvate removal by chick tissues. After the tissues had been aerobically shaken for two hours at 37°C. in the Barcroft differential manometers, the contents of the flasks containing tissues with no added pyruvate, with added pyruvate, and pyruvate plus vitamin were transferred to 5 cm. mortars to which had previously been added 1 cc. of 20 per

cent trichloroacetic acid. At the same time the contents of two control flasks containing the same amount of pyruvate as had been added to the experimental flasks, but no tissue, were washed into 15 cc. graduated tubes containing 2 cc. of 20 per cent trichloroacetic acid, made to volume, and later analyzed with the others in order to determine the original concentration of added pyruvate in the experimental flasks. The tissues were thoroughly ground in the small mortars with glass pestles and washed into 15 cc. graduated centrifuge tubes. After diluting to volume and mixing, the tubes were allowed to stand for one-half hour and then centrifuged. Aliquots from the centrifugates were transferred to 50 cc. Erlenmeyers, brought to pH 2.0 with sodium hydroxide, and treated with sodium bisulphite. It was found that further washing of the tissue residue with trichloroacetic acid was unnecessary. The bisulphite-binding capacity was then determined by titration with N/200 iodine according to the procedure of Clift and Cook (5).

(1 ml. N/200 I = 0.22 mgm. pyruvic acid)

RESULTS. It was found that pyruvate was readily utilized as substrate for respiration both by normal and polyneuritic chick cerebrum. Although the no-substrate level of respiration is not significantly reduced in avitaminous cerebrum, as may be seen in table 1, the increase in oxygen uptake produced by adding pyruvate was not as great in avitaminous as in normal cerebrum. This subnormal rate of respiration in the vitamin B₁ deficient cerebrum was brought back nearly to the normal level by the *in vitro* addition of 2.5 gamma of crystalline vitamin B₁. The increased oxygen uptake produced by the added vitamin is especially significant in the second and third 40 minute periods of respiration. In the absence of added vitamin the respiration of avitaminous cerebrum in pyruvate substrate steadily falls off during the course of the experiment. But when added vitamin B₁ is present the respiration of cerebrum in pyruvate is maintained at a level which approaches that of normal tissue.

In working with kidney special precautions had to be taken to use highly purified pyruvic acid. When a neutralized solution of pyruvate which had stood for over a week at 0° was tested, marked loss in bisulphite-binding capacity, sometimes as much as 40 per cent, was observed. Such pyruvate, when used as substrate for avitaminous kidney in concentrations greater than 0.005M produced definite inhibition of respiration, and additions of vitamin B₁ brought back the lowered respiration to a no-substrate level. Impure pyruvate had no effect upon normal kidney respiration. These results, which have been repeatedly observed, indicate that pyruvic acid containing appreciable amounts of higher polymers is unsuitable for use as tissue substrate. The impurities of pyruvic acid are especially toxic to avitaminous kidney and are rectified by additions of vitamin B₁. How-

TABLE 1

Effect of vitamin B₁ upon the oxygen uptake of avitaminous cerebrum as compared with normal cerebrum in pyruvate

P = pyruvate; V = vitamin B₁ 2.5 gamma. Expressed as cmm. O₂/gm./hr. (wet weight) (N. T. P.).

EXPERIMENT	SUBSTRATE	CONCENTRATION OF PYRUVATE IN RESPI- ROMETERS	PERIODS (MINUTES)			TOTAL. CCM./ GM./2 HRS.
			0-40	40-80	80-120	
Avitaminous cerebrum						
100	—	—	930	751	597	1,519
	P	0.012 M	1,410	1,257	1,023	2,460
	P + V	0.012 M	1,638	1,451	1,268	3,015
101	—	—	1,125	771	551	1,632
	P	0.017 M	1,609	1,188	885	2,460
	P + V	0.017 M	1,666	1,420	1,322	2,781
102	—	—	996	582	447	1,330
	P	0.016 M	1,522	1,098	1,030	2,440
	P + V	0.016 M	1,717	1,213	1,226	2,800
103	—	—	871	691	586	1,419
	P	0.017 M	1,551	1,285	1,057	2,599
	P + V	0.017 M	1,470	1,468	1,217	2,772
104*	—	—	1,000	760	546	1,540
	P	0.015 M	1,720	1,410	960	2,797
	P + V	0.015 M	1,690	1,593	1,125	2,926
Ave.	—		984	711	545	1,488
	P		1,562	1,247	991	2,551
	P + V		1,636	1,429	1,232	2,859
Normal cerebrum						
105	—	—	1,197	792	615	1,735
	P	0.011 M	1,670	1,604	1,377	3,246
106	—	—	996	709	477	1,448
	P	0.021 M	1,769	1,665	1,510	3,300
107	—	—	625	684	552	1,350
	P	Not determined	1,756	1,620	1,358	3,105
108	—	—	940	812	517	1,524
	P	0.032 M	1,840	1,734	1,545	3,425
Ave.	—		939	749	540	1,514
	P		1,758	1,656	1,448	3,294

(Ringer's phosphate-pyrophosphate used throughout.)

* Mild case of polyneuritis.

TABLE 2

Effect of vitamin B₁ upon the oxygen uptake of avitaminous kidney as compared with normal kidney in pyruvate

P = pyruvate; V = vitamin B₁ 2.5 gamma. Expressed as cmm. O₂/gm./hr. (wet weight) (N. T. P.).

EXPERIMENT	SUBSTRATE	CONCENTRATION OF PYRUVATE IN RESPIROMETERS	PERIODS (MINUTES)			TOTAL, CMM./ GM./2 HRS.
			0-40	40-80	80-120	
Avitaminous kidney						
110	—	—	1,751	1,561	1,358	3,110
	P	0.0038 M	1,857	1,833	1,649	3,558
	P + V	0.0038 M	1,933	1,923	1,707	3,708
	P	0.0076 M	1,848	1,789	1,690	3,524
	P + V	0.0076 M	2,197	2,161	1,927	4,200
111	—	—	1,600	1,559	1,200	2,900
	P	0.0038 M	1,743	1,864	1,624	3,485
	P + V	0.0038 M	1,885	2,114	1,670	3,770
	P	0.0076 M	1,870	2,032	1,723	3,735
	P + V	0.0076 M	1,998	2,245	1,839	4,060
	P	0.015 M	1,614	1,747	1,488	3,241
112	—	—	1,479	1,479	1,201	2,778
	P	0.0076 M	1,400	1,475	1,270	2,760
	P + V	0.0076 M	1,652	1,808	1,571	3,350
113	—	—	1,827	1,503	1,387	3,140
	P	0.0042 M	1,581	1,441	1,398	2,938
	P + V	0.0042 M	1,962	1,729	1,719	3,615
Ave.	—		1,664	1,525	1,286	2,982
	P		1,718	1,739	1,559	3,333
	P + V		1,938	1,980	1,756	3,951
Normal kidney						
121	—	—	1,810	1,604	1,418	3,200
	P	0.0038 M	2,350	2,340	2,042	4,480
122	—	—	1,690	1,611	1,390	3,130
	P	0.0076 M	2,645	2,630	2,390	5,120
123	—	—	2,080	1,722	1,453	3,502
	P	0.0044 M	2,142	2,081	1,977	4,140
124	—	—	1,975	1,810	1,501	3,520
	P	0.015 M	2,532	2,446	2,404	4,970
Ave.	—		1,881	1,687	1,443	3,338
	P		2,417	2,374	2,203	4,678

ever, pyruvate from which the impurities were removed by repeated vacuum distillation was readily utilized by normal cerebrum and kidney. The respiration of kidney was markedly influenced by the concentration of pyruvate used. Maximum stimulation was produced by low concentrations of pyruvate. The effect of variations in pyruvate concentration is given in experiment 111, table 2. It may be seen that 0.0038M and 0.0076M pyruvate produced stimulation to oxygen uptake of avitaminous kidney; but when the pyruvate concentration was increased to 0.015M the oxygen uptake was only slightly greater than the no-substrate uptake. This same concentration of pyruvate was readily utilized by normal kidney as is shown by experiment 124. Thus, there is a lowered pyruvate tolerance in avitaminous kidney. Table 2 also shows that the lower concentrations of pyruvate are not readily utilized by avitaminous kidney. In some cases the addition of pyruvate produced no increase in avitaminous

TABLE 3

Effect of vitamin B₁ upon aerobic removal of added pyruvic acid by avitaminous cerebrum
Pyruvic acid removal is given as mgm./gm. fresh tissue/2 hrs.

EXPERIMENT	INITIAL CONCENTRATION, MGM./GM. TISSUE	ACTUAL REMOVAL			THEORETICAL REMOVAL. EFFECT OF VITAMIN (FROM EXTRA O ₂ UPTAKE)
		Without vitamin	With vitamin	Effect of vitamin (difference)	
109	32.0	11.21	15.21	4.00	0.04
101	28.4	7.23	10.78	3.55	0.42
103	27.8	6.11	6.99	0.88	0.27
102	27.0	3.98	5.90	1.92	0.57
104*	25.3	6.19	6.77	0.58	0.21
100	20.2	3.61	7.81	4.20	0.87

* Mild case of polyneuritis.

kidney respiration. However, the oxygen uptake of normal kidney was greatly increased by pyruvate additions. When 2.5 gamma of vitamin were added to avitaminous kidney its respiration in pyruvate substrate was always improved, but was not restored to the normal level. We have also made additions of vitamin to avitaminous kidney in the absence of added substrate, but obtained no significant increases in oxygen uptake. Likewise, additions of vitamin B₁ to normal kidney with and without added pyruvate were without effect.

In order to see whether the extra oxygen uptake obtained with added vitamin B₁ was accompanied by an increased removal of pyruvate, the method of Clift and Cook (5) was applied to the contents of the respirometer flasks after shaking for two hours. The method followed in the treatment of the tissues for analysis is described above. The results obtained with avitaminous cerebrum are given in table 3.

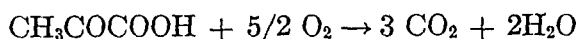
It will be seen that with the exception of experiment 104, in which case the chick exhibited only mild symptoms of polyneuritis, the removal of pyruvate was proportional to the concentration of the pyruvate. In all cases the amount of pyruvate removed by avitaminous cerebrum was increased by adding vitamin B₁ to the tissue. Variations in the amount of pyruvate removed by the vitamin appear to be due to variations in the severity of the polyneuritis. Although we never use chicks in these studies until they exhibit head retractions and are unable to walk, there are still variations depending upon the length of time they have been in this condition. A greater vitamin effect is always obtained with a more severe case of polyneuritis. The theoretical amount of pyruvate oxidatively removed by the vitamin was calculated from the extra oxygen uptake produced by

TABLE 4

Effect of vitamin B₁ upon aerobic removal of added pyruvic acid by avitaminous kidney
Pyruvic acid removal is expressed as mgm./gm. fresh tissue/2 hrs.

EXPERIMENT	INITIAL CONCENTRATION, MGM./GM. TISSUE	ACTUAL REMOVAL			THEORETICAL REMOVAL. EFFECT OF VITAMIN (FROM EXTRA O ₂ UPTAKE)
		Without vitamin	With vitamin	Effect of vitamin (difference)	
115	38.8	6.35	6.73	0.38	0.43
117	32.0	6.08	8.26	1.18	0.76
116	15.5	6.58	7.29	0.71	0.84
113	10.0	5.01	6.27	1.26	1.06
114	7.6	3.70	4.45	0.75	0.45

the vitamin. The assumption is made that the pyruvate is completely oxidized to CO₂ and H₂O according to the following equation:



The extra oxygen uptake was in no case sufficient to account for all of the pyruvate removed. It was thought that there might be some loss of pyruvate due to an acid-stable combination of its carbonyl group with some binding group present in the tissue; but experiments run under anaerobic conditions showed that there was no removal of added pyruvate in the absence of oxygen by either normal or avitaminous cerebrum and kidney. Lactic acid determinations have also been made upon tissues with added pyruvate with and without added vitamin, but no indications for a reduction of pyruvic acid to lactic acid were obtained. It is probable that the pyruvate is incompletely oxidized or a portion of it is removed by aerobic synthesis.

Increased removal of pyruvate by added vitamin was also found in avitaminous kidney as is shown in table 4. But in kidney better agreement

was obtained between the actual and the theoretical effect of the vitamin upon pyruvate removal.

DISCUSSION. Although these experiments with polyneuritic chick cerebrum and kidney demonstrate that vitamin B₁ is concerned in the oxidative metabolism of pyruvic acid, the elucidation of the exact stage of its oxidation effected by vitamin B₁ and the mechanism of the reaction await further investigations. A comparison of these results with our earlier results obtained with lactic acid substrate indicates that in chicks the vitamin is more closely associated with pyruvate than with lactate, since we were unable to obtain any vitamin effect in cerebrum using lactate substrate. With pyruvate substrate vitamin effects in cerebrum as well as kidney were consistent. In pigeon brain Peters and his co-workers have obtained an effect of the vitamin upon respiration both in lactate and pyruvate, and have found the two acids interchangeable. However, Meikeljohn (8) showed that the extra oxygen uptake produced by adding vitamin to pigeon cerebrum in lactate substrate was not accompanied by an increased removal of lactate. It appears that the vitamin is concerned with pyruvate removal, and that apparent vitamin effects upon respiration in lactate substrate may be secondary to its action in the removal of pyruvate, since small amounts of pyruvate have been shown to exert an inhibitory action upon lactic acid dehydrogenase activity of tissues. However, the fact that the addition of vitamin B₁ alone does not completely restore the utilization of pyruvate by avitaminous tissue to a normal level suggests that either there is a loss of some other component besides vitamin B₁ necessary for pyruvate metabolism, or that the effects here observed are still secondary to the participation of the vitamin in some other reaction closely associated with pyruvate oxidation.

SUMMARY

1. The oxygen uptake of minced cerebrum and kidney from normal and polyneuritic chicks in pyruvate substrate was studied. The effect of added vitamin B₁ upon pyruvate oxidation in avitaminous cerebrum and kidney was determined by means of oxygen uptake studies and chemical determinations of pyruvate removal.

2. Respiration of vitamin B₁ deficient cerebrum in pyruvate is sub-normal. The ability of chick kidney to utilize pyruvate substrate for respiration is seriously impaired in polyneuritis.

3. The oxygen uptake of avitaminous cerebrum and kidney in pyruvate substrate is increased to a nearly normal level by *in vitro* additions of small amounts (2.5 gamma) of vitamin B₁.

4. This extra oxygen uptake produced by added vitamin B₁ to avitaminous tissues is accompanied by an increased removal of pyruvate.

REFERENCES

- (1) PETERS, R. A., H. RYDIN AND R. H. S. THOMPSON. *Biochem. J.* **29**: 53, 1935.
- (2) SHERMAN, W. C. AND C. A. ELVEHJEM. *Biochem. J.* **30**: 785, 1936.
- (3) THOMPSON, R. H. S. AND R. E. JOHNSON. *Biochem. J.* **29**: 694, 1935.
- (4) JOHNSON, R. E. *Biochem. J.* **30**: 31, 1936.
- (5) CLIFT, F. P. AND R. P. COOK. *Biochem. J.* **26**: 1788, 1932.
- (6) PETERS, R. A. AND R. H. S. THOMPSON. *Biochem. J.* **28**: 916, 1934.
- (7) CASE, E. M. *Biochem. J.* **26**: 753, 1932.
- (8) MEIKELJOHN, A. P. *Biochem. J.* **27**: 1310, 1933.

A STUDY OF ANAEROBIC GLYCOLYSIS IN TISSUES FROM POLYNEURITIC CHICKS¹

THE NEGATIVE ACTION OF VITAMIN B₁

W. C. SHERMAN AND C. A. ELVEHJEM

From the Department of Agricultural Chemistry, Madison, Wisconsin

Received for publication June 8, 1936

It has been demonstrated in previous publications (1, 2) that in polyneuritic chicks there are abnormalities in the oxidative metabolism of lactic acid and to a greater extent of pyruvic acid which can be largely alleviated by the *in vitro* addition of vitamin B₁ to the avitaminous tissues. Although these results can all be adequately explained on the basis that vitamin B₁ functions in the oxidative removal of these intermediates in carbohydrate breakdown, it seemed possible that in the absence of vitamin B₁ there might also be abnormalities in anaerobic glycolysis of tissues. Although Neuberg and his school advocate methylglyoxal as an intermediate in the formation of lactic acid from carbohydrate, Embden and his co-workers (3) and Meyerhof and Keissling (4) found that in muscle pyruvic acid is the precursor of lactic acid.

Geiger (5), in a study of the rôle of glutathione in anaerobic tissue glycolysis, suggests that lactic acid production from glucose may pass through the intermediate methylglyoxal, but that the breakdown of glycogen to lactic acid follows the Embden-Meyerhof scheme which involves the intermediate pyruvic acid. He found that reduced glutathione was essential in the conversion of glucose and methylglyoxal into lactic acid, but not in the breakdown of glycogen to lactic acid.

Anaerobic glycolysis was, therefore, studied in polyneuritic chick tissues with and without added vitamin B₁ in order to determine whether or not the vitamin was necessary in the anaerobic formation of lactic acid. We were primarily interested in determining the amount of pyruvic and lactic acid which accumulated under these conditions.

The results obtained from these experiments show that avitaminous tissues readily form lactic acid (from glucose and glycogen) under anaerobic conditions and that added vitamin B₁ is not necessary in the anaerobic conversion of pyruvic acid into lactic acid.

¹ Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

METHODS. Tissues from large polyneuritic chicks (200–400 grams) were used in these studies. The chicks were killed by decapitation and the organs to be studied were immediately removed and placed upon filter papers where they were carefully wiped free from excess blood. The tissues were then placed upon watch glasses and finely minced with scissors. Portions of the tissue mince were weighed on pieces of cover slip and introduced into Thunberg tubes containing 4 cc. of buffer solution (ringer phosphate

TABLE 1

Anaerobic glycolysis of B₁ deficient chick tissues. The negative effect of added vitamin B₁

TISSUE	BUFFER	SUBSTRATE	DISULPHITE- BIND- ING SUBSTANCES CALCULATED AS MGM. PYRUVIC ACID PRODUCED BY 100 GRAMS TISSUE PER HOUR		MGM. LACTIC ACID PRODUCED BY 100 GRAMS TISSUE PER HOUR	
			No vita- min	Added vitamin	No vita- min	Added vitamin
Cerebrum	Ringer phosphate		2.5	2.5 (4 γ)	32	31 (4 γ)
Cerebrum	Ringer phosphate	Glucose 12 mgm.	3	3 (4 γ)	322	320 (4 γ)
Heart	Ringer phosphate		2	2.5 (4 γ)	50	50 (4 γ)
Heart	Ringer phosphate	Glucose 12 mgm.	4	3.5 (4 γ)	205	199 (4 γ)
Kidney	Ringer phosphate	Glucose 12 mgm.	13	15 (4 γ)	190	211 (4 γ)
Skeletal muscle	Ringer phosphate	Glucose 12 mgm.	1.5	2 (4 γ)	275	290 (4 γ)
Heart	Ringer phosphate	Glycogen 60 mgm.	6	5 (2 γ)	156	156 (2 γ)
Kidney	Ringer phosphate	Glycogen 60 mgm.	15	14 (2 γ)	141	149 (2 γ)
Liver	Ringer phosphate	Glycogen 60 mgm.	23	22 (2 γ)	68	68 (2 γ)
Skeletal muscle	Ringer phosphate	Glycogen 60 mgm.	11	10 (2 γ)	340	339 (2 γ)
Heart	Ringer phosphate + pyrophosphate	Glycogen 60 mgm.	5	5 (5 γ)	149	153 (5 γ)
Kidney	Ringer phosphate + pyrophosphate	Glycogen 60 mgm.	18	19 (5 γ)	159	156 (5 γ)
Liver	Ringer phosphate + pyrophosphate	Glycogen 60 mgm.	16	19 (5 γ)	65	79 (5 γ)
Skeletal muscle	Ringer phosphate + pyrophosphate	Glycogen 60 mgm.	9	10 (5 α)	482	494 (5 γ)

\pm pyrophosphate), pH 7.3. A measured amount (1 cc.) of an aqueous solution of substrate was pipetted into the bulb of the Thunberg tubes. The vitamin B₁ concentrate used was Oryzanin Fortior "Sankyo" obtained from the Takamine Corporation. The vitamin solution replaced an equivalent amount of buffer solution in the tubes to which vitamin additions were made. The tubes were then evacuated and placed in a constant-temperature bath maintained at 37°C. The substrates were mixed with

the tissues and incubated at 37°C. for a definite length of time, during which the tubes were frequently shaken by hand. At the end of the incubation period the tubes were opened and 2 cc. of 20 per cent trichloroacetic acid were added immediately. The contents of the tubes were then washed into small mortars where the tissue particles were thoroughly ground. The solutions were then transferred to 15 cc. centrifuge tubes, made to volume, and centrifuged. Measured aliquots were analyzed for pyruvic acid according to the method of Clift and Cook (6) and for lactic acid according to the method of Friedemann and Graesser (7).

The glycogen used in these experiments was prepared from fresh beef liver according to the method of Sahyun and Alsberg (8). This method of preparation is rapid, requires no alkaline digestion, and yields a product of excellent quality.

RESULTS. The values obtained with various chick tissues are shown in table 1. The results show that avitaminous tissues readily form lactic acid from glucose and glycogen under anaerobic conditions. Vitamin B₁ added to avitaminous tissues has no apparent effect upon tissue glycolysis. There is no evidence of an accumulation of pyruvic acid under these conditions. It is of interest to note that of the tissues studied, kidney and liver have by far the highest content of bisulphite-binding substances. In these organs, values of 15 to 20 mgm. pyruvic acid/100 gm/hr. were found, whereas in other organs the values range from 2 to 5. Skeletal muscle appears to be intermediate in this respect. But the addition of vitamin B₁ has no effect upon this phenomenon.

DISCUSSION. These experiments show that anaerobic glycolysis readily takes place in tissues from polyneuritic chicks. Abnormalities in pyruvate metabolism, which have been previously described and shown to be closely associated with vitamin B₁, cannot be attributed to faulty glycolysis in avitaminosis B₁. There is no evidence that vitamin B₁ has a function in anaerobic carbohydrate metabolism. Its action appears to be limited to the oxidative metabolism of these carbohydrate intermediates.

SUMMARY

1. Anaerobic glycolysis was studied in vitamin B₁-deficient chick cerebrum, heart, kidney, liver, and skeletal muscle. The effect of *in vitro* additions of vitamin B₁ upon the anaerobic formation of lactic and pyruvic acid from glucose and glycogen was determined.
2. Avitaminous tissues readily form lactic acid from glucose and glycogen under anaerobic conditions.
3. There is no significant accumulation of pyruvic acid anaerobically.
4. Added vitamin B₁ has no effect on anaerobic glycolysis.

REFERENCES

- (1) SHERMAN, W. C. AND C. A. ELVEHJEM. *Biochem. J.* **30**: 785, 1936.
- (2) SHERMAN, W. C. AND C. A. ELVEHJEM. *This Journal* **117**: 142, 1936.
- (3) EMBDEN, G., H. J. DEUTICKE AND G. KRAFT. *Klin. Wehnschr.* **12**: 213, 1933.
- (4) MEYERHOF, O. AND W. KEISSLING. *Biochem. Ztschr.* **264**: 60, 1933.
- (5) GEIGER, A. *Biochem. J.* **29**: 811, 1935.
- (6) CLIFT, F. P. AND R. P. COOK. *Biochem. J.* **26**: 1788, 1932.
- (7) FRIEDEMANN, T. E. AND J. B. GRAESER. *J. Biol. Chem.* **100**: 291, 1933.
- (8) SAHYUN, M. AND C. L. ALSBERG. *J. Biol. Chem.* **89**: 33, 1930.

FURTHER STUDIES ON THE EFFECTS OF NaF ADMINISTRATION UPON THE BASAL METABOLIC RATE OF EXPERIMENTAL ANIMALS¹

PAUL H. PHILLIPS

From the Department of Agricultural Chemistry, Madison, Wisconsin

Received for publication June 12, 1936

The level of administration of NaF in the studies reported by Phillips et al. in 1935 was three or more times that suggested for the control of thyrotoxicosis by Goldemberg in 1932. It seemed that the level of feeding used might have been too high to produce the reduction in the metabolic rate claimed by Goldemberg. Since the previous studies did not duplicate the exact dosage used by Goldemberg, a second series of studies was undertaken with a view of extending the results previously obtained.

Accordingly, an experiment was planned to determine the effects of the mode of administration, the dosage, and the element of time in an attempt to influence the metabolic rate of experimental animals by the use of NaF. Further, it seemed advisable to determine if previous sensitization to desiccated thyroid influenced the subsequent action of NaF. Data were also desirable in more than a single species. Guinea pigs were employed in one experiment and data were obtained as in the case of the rat with the additional study of the effects of scurvy on the BMR.

EXPERIMENTAL. Vigorous mature male rats were used in one experiment. As in the former case, the basal ration A described by Lamb et al. in 1933 was used. When fluorine was given it was injected intraperitoneally in a solution whose concentration was 0.9 per cent and at a rate of 18 mgm. of NaF per kilogram of body weight. This was the concentration and dosage favorably reported by Goldemberg in 1930 for the reduction of the normal metabolic rate of experimental animals. In the case of the guinea pigs fairly mature but still growing animals were used. A basal ration composed of rolled oats 68 parts, artificially dried alfalfa hay 20 parts, commercial casein 5 parts, yeast 5.5 parts, irradiated yeast 0.5 part, steamed bone meal 1 part, and iodized NaCl 0.5 was fed. This ration gave excellent results when supported by ample daily doses of orange juice (5 cc.) and it produced scurvy in 28 days when orange juice was withheld.

In each experiment the basal metabolic rates were obtained several times for each animal before subjecting it to experimental treatment. Thus, a

¹ Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

normal BMR was obtained for each individual. Subsequently the experiment was so arranged as to permit a weekly basal metabolism determination for each animal throughout each phase of the study. In this manner the performance of a single animal was directly comparable to its own normal record as well as to the record of any control animals throughout the experiment. The basal metabolic rates were determined as outlined by Phillips et al. in 1935. The body weights were recorded.

RESULTS. The body weights of the experimental animals present interesting results. Fully mature male rats weighing 350 to 400 grams each lost 15 to 30 per cent of their body weight when subjected to a weekly fast of 24 hours, duration and a ration containing 0.25 per cent desiccated thyroid. Withdrawal of the desiccated thyroid from the ration caused a prompt and steady increase in body weight until the original body weights were regained. The intraperitoneal injection of a single dose of NaF (18 mgm./kgm.) during the middle of the third week following the removal of the desiccated thyroid promptly arrested body weight recovery and caused a sharp loss almost equivalent to that lost during the desiccated thyroid feeding period. In younger male rats (250 grams) little loss of weight was caused by feeding 0.25 per cent desiccated thyroid in the ration. However, a single intraperitoneal injection of NaF during the third week after cessation of desiccated thyroid feeding produced the same response as recorded for the older and heavier animals. The continuous daily intraperitoneal injections of 18 mgm. of NaF per kilogram of body weight and a weekly 24 hour fast caused a variable reaction upon body weight during the first week. Thereafter there was a gradual and continuous loss in body weight, amounting to 14 or 15 per cent in 28 to 35 days at which time the injections were discontinued.

The intraperitoneal injection of NaF (18 mgm./kgm.) in the guinea pigs seemed to have little influence upon growth. When the same animals were changed from a daily injection of 18 mgm. of NaF per kilogram of body weight to the administration of an oral dose of 50 to 60 mgm. of NaF fed in solution by pipette, growth was arrested during the third week. A sharp and rapid loss of body weight occurred, which amounted to 35 or 40 per cent. The weight records of the scorbutic animals were typical.

Table 1 shows the effect of NaF administration upon the basal metabolic rate. The method used for making the BMR determinations has repeatedly given consistent duplicate results with a variability within ± 7 per cent. A variation of 10 per cent should permit a conservative limit for comparison of results between weekly determinations. With this as a guide it seems that continuous daily injection of 18 mgm. of NaF per kilogram of body weight may cause a slight rise in the metabolic rate of the rat in 28 days. No change was noted in the case of the guinea pig. When their dose of NaF was changed to an oral dose of 50 to 60 mgm. of NaF per

kilogram of body weight no pronounced change was noted although a tendency to increase was indicated by the 28th day. The route of administration or the dosages used had no effect upon the normal metabolic rate of these animals.

TABLE 1

The effect of NaF administration upon the basal metabolic rate
(Cal. per sq. m. of body surface per day)

	RAT 1	RAT 2	GUINEA PIG 1	GUINEA PIG 2
Basal rate				
	694	640	775	754
NaF injected intraperitoneally (18 mgm./kgm. of body weight)				
2.4 hours.....	580		650	732
7th or 14th day.....	770	623	795	690
7th-14th day 2 hours after injection.....	727	600	856	775
28th day.....	806	746	785	815
NaF fed (50.60 mgm./kgm. of body weight)				
14th day.....			710	607
21st day.....			728	608
28th day.....			859	

TABLE 2

The effect of previous desiccated thyroid feeding upon the reaction to fluoride administration

(Cal. per sq. m. of body surface)

	RAT 1	RAT 2	RAT 3	RAT 4	RAT 5	RAT 6
Basal rate						
	634	718	722	710	828	855
Desiccated thyroid feeding (0.25 per cent level)						
14th day.....	1,280	1,576	1,365	1,640	610	1,400
28th day.....	1,415	875	2,015	2,000		
Returned to basal ration without desiccated thyroid						
21st day.....	744	614	783	970	750	740
21st day—2 hours after NaF injection	985	945			750	832
55th day.....		670	792	705		756
55th day—2 hours after NaF injection		605	705	645	705	730

Table 2 shows that the feeding of 0.25 per cent of desiccated thyroid to the rat causes an increase in the metabolic rate by the 14th day of feeding and this generally continued to mount to the 28th. In this experiment the BMR had returned to normal 21 days after the end of the 4-week desic-

cated thyroid feeding period. At this time the basal rates were distinctly raised by the intraperitoneal injection of NaF in 3 cases out of 4. This indicates that a residual desiccated thyroid effect was still present. This reaction was lost by the 55th day following the removal of desiccated thyroid from the ration. The results seem to indicate a small and uniform lowering of the basal metabolic rates of all of the animals on the 55th day following the injection of NaF. These results, however, lie within the limits of ± 10 per cent of their original basals and are unlikely to be of special significance. The data in table 2 are interpreted to mean that the administration of NaF does not lower the basal metabolic rate of the rat following the feeding of desiccated thyroid once the effect of the latter has completely vanished. NaF seems to augment the action of the desiccated thyroid so long as a residual sensitization to desiccated thyroid is present

TABLE 3

The effect of scurvy upon basal metabolism
(Cal. per sq. m. of body surface)

	ANIMAL 3	ANIMAL 4	
	Basal rate		
	812	780	
Scurvy ration			
14th day.....	780	743	
21st day.....	773	680	Growth arrested
28th day.....	859	971	Typical scurvy
35th day.....	725	830	After 10 cc. of O. J. on 28th day
35th day.....	960	825	2 hours after NaF injection
42nd day.....	778	740	5 cc. of O. J. on 35th day
54th day.....	786	800	Severe chronic scurvy

in the body. Feeding 0.20 per cent desiccated thyroid caused death in two guinea pigs on the 7th day.

In table 3 the effects of the development of scurvy and the basal metabolic rate are recorded. There is a tendency for the basal metabolic rate to increase when the deficiency approaches the stage of typical scorbutic symptoms. It is to be noted that a definite increase is recorded in both cases. In one of these cases the increase exceeded the original basal rate by more than 10 per cent. An attempt was made to maintain these animals and obtain another record in typical scurvy but it was unsuccessful. The feeding of single doses of orange juice caused the basal metabolic rate to resume its normal level. When NaF was injected intraperitoneally on the 35th day when the animals were in sub-acute scurvy, variable results were secured. A rise was recorded in one case and none in the other.

SUMMARY AND CONCLUSIONS

The administration of NaF has been found to be without effect upon the basal metabolic rate in the normal experimental animal. No difference in action was noted regardless of whether 18 mgm. of NaF per kilogram of body weight were injected intraperitoneally or were fed orally at the rate of 50 to 60 mgm. of NaF per kilogram of body weight. Thus, the route of administration or the dosage has no influence upon the results obtained. Time, as evidenced by daily intraperitoneal injections, was without influence from 2 hours after the injection up to 28 days, although in the latter case a slight increase was recorded. Previous sensitization with desiccated thyroid failed to produce an effect after 21 days. NaF injections during the period of desiccated thyroid sensitivity caused a rise in the metabolic rate.

The metabolic rate tended to rise in the condition of scurvy, or when NaF was injected in sub-acute scurvy. The results were variable and inconclusive.

The daily intraperitoneal injection of a solution of NaF at the rate of 18 mgm. per kilogram of body weight caused a loss of body weight in the rat which amounted to 14 or 15 per cent. This was closely parallel to the loss of body weight caused by feeding 0.25 per cent of desiccated thyroid. The body weight of guinea pigs was not greatly influenced by the level of 18 mgm. of NaF injected daily, but was sharply reduced by feeding 50 to 60 mgm. of NaF per kilogram of body weight. Two-tenths per cent desiccated thyroid proved fatal to guinea pigs in 7 days.

These results with rats and guinea pigs do not confirm the published work of Goldemberg. Insofar as previous sensitization to desiccated thyroid simulates a condition of thyrotoxicosis, there is no evidence from these experiments to indicate that it could be controlled by NaF feeding. Neither has it been possible to reduce the normal metabolic rate of experimental animals by NaF administration under a variety of conditions.

REFERENCES

- GOLDEMBERG, L. De Buenos Ayres La Semana Med. **360**: 1639, 1932.
J. de Physiologie et de Path. Generale **28**: 556, 1930.
LAMB, A. R., P. H. PHILLIPS, E. B. HART AND G. BOHSTEDT. This Journal **106**: 350, 1933.
PHILLIPS, P. H., H. E. ENGLISH AND E. B. HART. This Journal **113**: 441, 1935.

THE RELATION OF PANCREATIC JUICE TO PANCREATIC DIABETES¹

HERMAN P. HARMS, JOHN VAN PROHASKA AND LESTER R. DRAGSTEDT

From the Department of Surgery of The University of Chicago

Received for publication April 30, 1936

Ever since the discovery by von Mering and Minkowski (1890) that extirpation of the pancreas in the dog produces a condition closely resembling diabetes mellitus in man, reports have appeared in the literature implicating the external secretion of the pancreas in this condition. In the intervening years much evidence has accumulated which indicates that the acinar tissue and presumably its product, pancreatic juice, play no rôle in pancreatic diabetes, but that this condition is due to partial or complete insufficiency of islet function. This evidence need not be reviewed here. There appears to be substantial basis for the generally accepted view that insulin is a product of the pancreatic islets and probably specifically of the beta cells. The uncertainty rests with the possible inter-relationship between insulin and the external secretion, the involvement of the latter in human diabetes, and in the mechanism of insulin action. A diminution in the enzymatic activity of the pancreatic juice in human diabetes has been reported by Katsch and von Friedrich (1922), Jones, Castle, Mulholland and Barley (1925), Labbé, Nepveux, and Adlersberg (1925), and Gavrila and Paraschivesco (1926). La Barre and Destree (1928) and La Barre (1930) demonstrated by means of cross circulation experiments, in which the blood of the donor was circulated through the head only of the recipient, that hyperglycemia in the donor increased the volume and enzyme concentration of the pancreatic secretion in the recipient. Conversely hypoglycemia in the donor diminished the secretion of pancreatic juice in the recipient. Baxter (1932) reported that in rabbits the injection of insulin produced hypoglycemia and diminished the output of enzymes in the pancreatic juice. Conversely Babkin (1935) found that in the same animal hyperglycemia increased the output of enzymes in the pancreatic juice, the effect being upon the endings of the parasympathetic nerves since it was abolished by atropine.

W. N. Boldyreff and E. B. Boldyreff (1928) have proposed a novel theory of pancreatic diabetes which implicates the external secretion. This

¹ This work has been aided by a grant from the Douglas Smith Foundation for Research of the University of Chicago.

theory may be stated somewhat as follows. Insulin, absorbed into the blood, stimulates the secretion of pancreatic juice. This juice contains a glycolytic substance and its absorption from the small intestine is responsible for the insulin effect on the blood sugar. In support of this theory W. N. Boldyreff (1934) states that when he ligated the smaller pancreatic duct of the dog and used the larger one for an external fistula, the symptoms of diabetes appeared in 24 hours. When the ligature was removed from the smaller duct the severe diabetic symptoms decreased at once and when the larger duct was closed the animal completely recovered. "Therefore," he concludes, "removal from the animal or human body of the external secretion leads to diabetes, hence the direct conclusion to treat diabetes by the introduction of pancreatic juice." E. B. Boldyreff (1928) reports that "during the periods of pancreatic secretion the sugar content is lower than during intervals between periods" and "pancreatic juice secreted into the small intestine and absorbed by the blood is one of the chief factors responsible for the changes in blood sugar content."

The repeated observations in many physiological laboratories that the production of a pancreatic fistula in the dog does not cause glycosuria and diabetes might be thought to have settled this question. However, it must be conceded that the customary methods for making such fistulae in the dog do not deprive the animal of all pancreatic juice because of the uniform presence of one or more accessory ducts. The effect of feeding pancreatic juice on the course of pancreatic diabetes has not been adequately tested, probably because of the difficulty in securing sufficient amounts of the fresh secretion. The administration of the whole fresh gland or of artificial juice made by extracting the fresh gland does not answer the question since these products contain materials which are not present in pancreatic juice. The development by L. R. Dragstedt, Montgomery, and Ellis (1930) of a method for making a total external fistula of all pancreatic ducts in the dog and for collecting the entire secretion provided an opportunity for reinvestigating this supposed relation of pancreatic juice to pancreatic diabetes.

EXPERIMENTAL PROCEDURE. Two types of experiment were done. In one group of animals the effect of the total loss of pancreatic juice on the development of diabetes was observed, while in the other an examination was made to determine the effect of the oral administration of pancreatic juice after complete pancreatectomy. Complete external fistulae of the pancreatic ducts were made in dogs in the following manner. An isolated closed sac was prepared of that portion of the duodenum, immediately below the entrance of the bile duct, which receives the various pancreatic ducts. A gold-plated cannula was placed in this sac and led through a stab wound in the abdominal wall. The sac and cannula were carefully wrapped with omentum and an end to end anastomosis made between the proximal and distal duodenum (fig. 1). By this method all of the external

secretion of the pancreas was lost to the body but could be quantitatively collected and administered to other animals. The secretion was activated by the admixture of succus entericus provided by the short segment of duodenum. These animals secreted from 500 to 1,400 cc. of pancreatic juice per 24 hours on the standard diet. To counteract the effect of the extensive loss of base and to prevent death from dehydration and acidosis, daily intravenous injections of 700 to 1,000 cc. of Ringer's solution were given. Small amounts of calcium carbonate and sodium bicarbonate were administered daily by mouth to prevent the occurrence of gastric or duodenal ulcers (Matthews and L. R. Dragstedt, 1932). With these

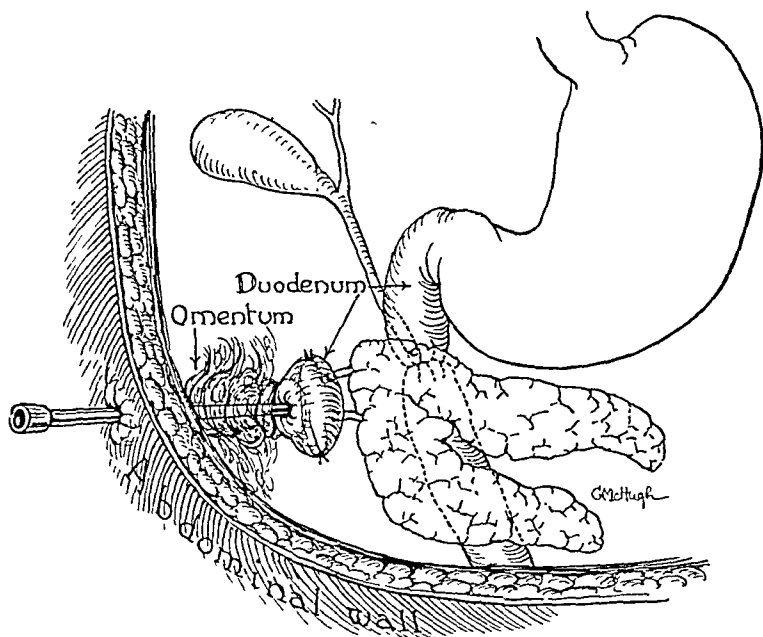


Fig. 1. Diagram of the method for making a total pancreatic fistula

precautions the animals, six in number, were kept in good condition for many weeks and provided a constant supply of active pancreatic juice. The urine of these dogs remained constantly free of sugar, except for an occasional sample usually immediately after operation when a trace of reducing substance was found. Three examinations of the fasting blood sugar in each animal were made at scattered intervals but all were well within the values found in normal dogs.

Healthy adult male dogs were completely depancreatized in a one-stage operation under ether anesthesia. After recovery each animal was placed on the standard diet consisting of 400 grams of meat, 400 cc. of whole milk, and 100 grams of white bread. Insulin was administered twice daily and an attempt was made to adjust the dosage so as to permit of only a

moderate glycosuria. The amount of sugar excreted in the urine was determined daily and occasional measurements were made of the blood sugar. Pancreatic juice was administered by stomach tube when not taken voluntarily. The freshly collected secretion only was used because of the well-known rapid deterioration of trypsin. In no instance was juice more than 24 hours old employed. The data on six depancreatized dogs are summarized in the following protocols.

PROTOCOLS. *Dog 1.* Weight 10.0 kgm. Pancreatectomy 1-31-35. After recovery the animal was placed on the standard diet and given 100 to 300 cc. of pancreatic juice daily. Fifteen to 18 units of insulin per day were given in three doses. During the first month the sugar excretion varied between 1.2 and 34 grams per day with an average of 6.4 grams. Conclusion: 300 cc. of pancreatic juice did not prevent or cure diabetes in this case.

Dog 2. Male. Weight 15.0 kgm. Pancreatectomy 2-1-35. After recovery this animal took the standard diet and from 75 to 300 cc. of pancreatic juice per day. During the first 18 days 15 units of insulin were given daily and the sugar excretion varied between 3 and 37 grams per day with an average of 13.5 grams. The insulin was then increased to 30 units and the sugar excretion decreased to an average of 5.1 grams per day for the following three weeks. Conclusion: 300 cc. of pancreatic juice did not prevent or cure diabetes in this case.

Dog 3. Male. Weight 6.7 kgm. Pancreatectomy 1-7-35. Standard diet and 200-500 cc. pancreatic juice daily. For the first month the daily sugar excretion averaged 7 grams on 15 to 20 units of insulin. Conclusion: 500 cc. of pancreatic juice did not prevent or cure diabetes in this case.

Dog 4. Male. Weight 16.8 kgm. Pancreatectomy 4-2-35. After recovery the animal took the standard diet and from 4-5-35 to 4-23-35 was given 500 to 800 cc. of pancreatic juice and 30 to 35 units of insulin. During this period the daily sugar excretion varied between 5 and 56 grams with an average of 11 grams. From 4-24-35 to 4-30-35 the juice was not given but the insulin and diet continued as before. During this period the daily sugar excretion varied between 2 and 11 grams with an average of 7.7 grams. From 5-1-35 to 5-19-35 from 600 to 800 cc. of pancreatic juice and 40 units of insulin were given daily and the sugar excretion averaged 9 grams. The juice was then withdrawn for 3 days and the sugar excretion promptly decreased, the urine becoming sugar-free on 5-22. On 5-23 and 5-24 there were severe hypoglycemia convulsions which were promptly relieved by glucose. From 5-28 to 6-16 from 800 to 1100 cc. of pancreatic juice and 40 units of insulin were given daily and the sugar excretion varied between 5 and 50 grams with an average of 26 grams per day. No juice was then given from 6-17 to 6-26 but the diet and insulin continued as before. The sugar excretion varied between 0 and 30 grams with an average of 8 grams.

Dog 5. Male. Weight 8.4 kgm. Pancreatectomy 5-9-35. After recovery this animal was given the standard diet and from 5-12 to 6-16 received 100 to 600 cc. of pancreatic juice and 10 units of insulin daily. During this period the sugar excretion varied between 0 and 4.8 grams per day with an average of 1.2 grams. From 6-17 to 7-1 the juice was discontinued and during this period the sugar excretion varied between 0 and 9 grams with an average of 2.0 grams. From 7-1 to 7-9 400 cc. of juice was given and the sugar excretion averaged 2.8 grams on the standard diet and 10 units of insulin daily.

Dog 6. Male. Weight 15.1 kgm. Pancreatectomy 6-20-35. After recovery the animal received the standard diet and 20 units of insulin daily but from 6-20 until

7-8 was given no pancreatic juice. During this period the sugar excretion varied between 0 and 35 grams with an average of 11.0 grams per day. From 7-9 to 7-16 400 cc. of pancreatic juice were given daily in addition to the same dose of insulin and the same diet. During this period the sugar excretion varied between 1.0 and 40.0 grams with an average of 18.0 grams. From 7-17 to 7-31 no pancreatic juice was given and the sugar excretion varied between 0 and 7 grams with an average 1.0 grams per day.

DISCUSSION. The results in both types of experiment were quite definite. In no instance did the continued total loss of pancreatic juice produce hyperglycemia or glycosuria in the dog. It is difficult to account for the reported findings of Boldyreff (1934) other than that this investigator must have confused the symptoms of dehydration and acidosis, resulting from the uncompensated loss of the alkaline pancreatic secretion, with diabetes. The data with respect to the feeding of pancreatic juice were equally clear cut. Depancreatized dogs 1, 2 and 3, receiving from 100 to 500 cc. of fresh pancreatic juice per day were in a somewhat better nutritional state than control animals, their stools were less bulky and more firm, but they remained diabetic and excreted large amounts of sugar on 15 to 30 units of insulin per day. The variation in the amount of sugar excreted by the depancreatized dog from day to day while on a constant diet and insulin intake was marked, in some cases amounting to as much as 45 grams. For this reason it was concluded that a comparison of the sugar excretion for short periods while pancreatic juice was administered would be misleading. In the protocols of dogs 4, 5 and 6, however, a comparison of periods of from 6 to 18 days was made, in each case a period of pancreatic juice feeding alternating with a control. The results were consistent throughout. In each case the administration of pancreatic juice not only did not decrease the sugar excretion but actually increased it. This effect would appear to be easily explained by the better digestion and absorption of glucose-forming substances in the intestine when pancreatic juice was given.

These observations are not out of harmony with the conclusions drawn by La Barre, Baxter, and Babkin from their experiments. Hyperglycemia may stimulate the secretion of pancreatic juice, but such increased secretion would not counteract the hyperglycemia if it were unaccompanied by an increased liberation of insulin into the blood stream.

SUMMARY

1. Total external pancreatic fistulae were prepared in six dogs. The complete withdrawal of pancreatic juice for 4 to 6 weeks did not cause hyperglycemia or glycosuria in these animals.

2. The oral administration of fresh dog pancreatic juice in amounts of from 300 to 1100 cc. per day to completely depancreatized dogs did not lessen the severity of the diabetes. On a standard diet and insulin intake,

the administration of pancreatic juice usually increased the glucose excretion.

REFERENCES

- BABKIN, B. P. J. A. M. A. 105: 1659, 1935.
 BAXTER, S. G. Quart. J. Exper. Physiol. 21: 355, 1932.
 BOLDYREFF, E. B. Pflüger's Arch. 218: 349, 1928.
 Bull. Battle Creek San. 24: 349, 1929.
 BOLDYREFF, W. N. Am. J. Digest. Dis. and Nutrition 1: 453, 1934.
 DRAGSTEDT, L. R., M. L. MONTGOMERY, AND J. C. ELLIS. Proc. Soc. Exper. Biol. and Med. 28: 109, 1930.
 GAVRILA, I. AND M. PARASCHIVESCO. Compt. rend. Soc. de Biol. 95: 761, 1926.
 JONES, C. M., W. B. CASTLE, H. B. MULHOLLAND AND F. BARLEY. Arch. Int. Med. 35: 315, 1925.
 KATSCH, G. AND L. VON FRIEDRICH. Klin. Wchnschr. 1: 112, 1922.
 LABARRE, J. This Journal 94: 17, 1930.
 LABARRE, J. AND P. DESTREE. Compt. rend. Soc. de Biol. 98: 1237, 1240; 99: 337, 1056, 1874, 1928; 101: 147, 1929.
 LABBÉ, M., F. NEPVEUX AND L. ADLERSBERG. Arch. d. mal de l'app. digestif 15: 871, 1925.
 MATTHEWS, W. B. AND L. R. DRAGSTEDT. Surg., Gynec. and Obst. 55: 265, 1932.
 VON MERING AND MINKOWSKI. Arch. f. exper. Path. u. Pharmacol. 26: 371, 1889-1890.

THE RELATION OF PANCREATIC JUICE TO THE FATTY INFILTRATION AND DEGENERATION OF THE LIVER IN THE DEPANCREATIZED DOG¹

JOHN VAN PROHASKA, LESTER R. DRAGSTEDT AND HERMAN P. HARMS

From the Department of Surgery of The University of Chicago

Received for publication May 4, 1936

Shortly after the discovery of insulin by Banting and Best (1922), it was noted by Fisher (1924) and by Allan, Bowie, Macleod and Robinson (1924) and others that completely depancreatized dogs adequately treated with insulin usually failed to survive more than two to three months. At death the most obvious change observed was an extensive fatty infiltration and degeneration in the liver. The addition of raw pancreas to the diet was found by the latter investigators to prevent the development of these liver changes and to permit survival for long periods of time. These findings have been abundantly confirmed. In 1930, Hershey, and in 1931, Hershey and Soskin, reported that the addition of 10 grams of lecithin daily to the diet of the depancreatized dog treated with insulin was also effective in preventing the liver damage and in permitting survival. The active constituent of lecithin in this effect was found by Best and Huntsman (1932) and Best, Ferguson and Hershey (1933) to be choline. Ralli, Flaun and Banta (1935) confirmed these observations with respect to lecithin, but concluded that it was not as effective as raw pancreas in preventing the deposition of liver fat. Berg and Zucker (1931) reported changes in the liver following pancreatic fistulae or ligation of the pancreatic ducts which they considered similar to those described by Fisher and by Allan, Bowie, Macleod and Robinson following pancreatectomy. They suggested that absence of the external pancreatic secretion from the intestine might be the common underlying factor in the three conditions.

The depancreatized animal clearly suffers from two known deficiencies, insulin and pancreatic juice. The fact that insulin together with an otherwise adequate diet does not suffice to permit the depancreatized animal to survive in good health, suggested that the pancreatic secretion might be of significance in this connection. The possibility that some of the pancreatic lipase might be absorbed into the blood and play a rôle in the migration of fat was considered by Allan, Bowie, Macleod, and Robinson, and led them

¹ This work has been aided by a grant from the Douglas Smith Foundation for Medical Research of The University of Chicago.

to investigate the effect of feeding raw pancreas. The following experiments were done to determine if the beneficial effect of raw pancreas administration was due to its content of the enzymes or other substances present in pancreatic juice.

a. *The effect of the total loss of pancreatic juice on the liver.* If the deficiency in depancreatized dogs which leads to fatty changes in the liver and ultimately to death were the absence of one or more of the constituents of pancreatic juice, as for instance lipase, in the intestinal tract, then such changes might be expected to develop after total pancreatic fistula. This should be true unless it were assumed that the responsible substance was absorbed directly into the blood as well as secreted with the pancreatic juice. Observations were made on seven dogs in which total pancreatic fistulae were prepared as described in our previous paper (Harms, Prohaska and Dragstedt, 1936). They were given a standard diet of meat, bread, and whole milk, and dehydration and acidosis were controlled by the intravenous injection of Ringer's solution and the oral administration of alkalies. They secreted from 500 to 1,400 cc. of pancreatic juice per day with an average daily secretion of about 750 cc. Two of the animals developed extensive subcutaneous abscesses and were sacrificed after 30 and 36 days respectively. In both of these animals a moderate fatty infiltration of the liver was demonstrated in microscopic sections (fig. 1). One animal died from peritonitis following rupture of the duodeno-pancreatic pouch 23 days after operation. In this animal also a slight to moderate fatty infiltration of the liver was found. The remaining four animals were free from infection when sacrificed 26, 35, 39 and 43 days after operation. In each case the liver was found to be entirely normal, both on gross and microscopic examination.

b. *The effect of ligation of the pancreatic ducts on the liver.* Complete obstruction to the pancreatic ducts not only prevents the entrance of pancreatic juice into the duodenum, but has been demonstrated to cause an extensive degeneration of the acinar tissue of the pancreas. The islet tissue in many cases remains sufficiently functional, however, to prevent the appearance of diabetes. If the deficiency in question were due to the absence of some substance manufactured by the acinar tissue and absorbed directly into the blood, fatty changes might be expected to develop in the liver of such animals. In three adult dogs the pancreatic ducts were ligated and divided, the pancreas separated from the duodenum and the omentum interposed between to prevent regeneration or anastomosis of the duct system and the duodenum. These animals were given a generous diet of lean meat, whole milk and bread, but they rapidly lost weight and died in 44, 56 and 83 days respectively. At autopsy all of the animals were emaciated and chronic gastric ulcers were found in two. The pancreas in each case was extensively degenerated but some acinar tissue as well as the

islets were preserved. Sections of the liver showed slight fatty infiltration in two cases (fig. 2), but in the third the liver was entirely normal.

c. *Effect of the oral administration of pancreatic juice on the development of fatty degeneration and infiltration of the liver in depancreatized dogs.* Nine adult male dogs were completely depancreatized in a one-stage operation under ether anesthesia. Immediately after recovery they were placed on the standard diet consisting of 400 grams of meat, 100 grams of bread and

Fig. 1. Photomicrograph showing the slight degree of fatty infiltration in the liver of a dog who had a total pancreatic fistula for 36 days. Stain Sharlach R. Magnification $\times 235$.

Fig. 2. Photomicrograph showing the slight degree of fatty infiltration in the liver of a dog whose pancreas had become extensively degenerated as a result of ligation of the pancreatic ducts. Stain Sharlach R. Magnification $\times 235$.

Fig. 3. Photomicrograph showing the extreme degree of fatty infiltration and degeneration in the liver of a depancreatized dog treated with insulin and given 1000 cc. of pancreatic juice daily. Death occurred in 65 days. Specimen taken at autopsy. Stain Sharlach R. Magnification $\times 235$.

Fig. 4. Photomicrograph showing the extreme degree of fatty infiltration and degeneration in the liver of a depancreatized dog treated with insulin and given 800 cc. of pancreatic juice daily. Death occurred 75 days after the pancreatectomy. Stain hematoxylin and eosin. Magnification $\times 235$.

Fig. 5. Photomicrograph of a biopsy of the liver of a depancreatized dog taken 30 days after the pancreatectomy and showing moderate fatty infiltration. Stain Sharlach R. Magnification $\times 375$.

Fig. 6. Photomicrograph of a biopsy of the liver of the same animal as used in figure 5 and showing the curative effect of feeding 25 grams of raw pancreas daily for one month. Stain Sharlach R. Magnification $\times 375$.

Fig. 7. Photomicrograph of a biopsy of the liver of the same dog used in figure 6 and showing the return of fat in the liver after feeding only 10 grams of raw pancreas per day for one month. Stain Sharlach R. Magnification $\times 235$.

Fig. 8. Photomicrograph of a biopsy of the liver of a depancreatized dog treated with insulin and showing definite fatty infiltration one month after the pancreatectomy. Stain hematoxylin and eosin. Magnification $\times 235$.

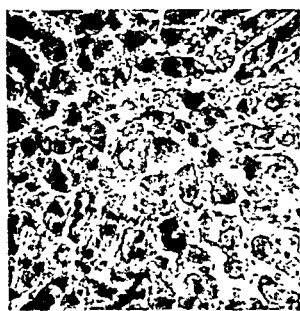
Fig. 9. Photomicrograph of a biopsy of the liver of the same dog used in figure 8 and showing an increase in the severity of the liver damage after the oral administration of 700 mgm. of choline chloride daily for 26 days. Stain hematoxylin and eosin. Magnification $\times 235$.

Fig. 10. Photomicrograph of a biopsy of the liver of a depancreatized dog showing definite fatty infiltration 22 days after the pancreatectomy. Stain Sharlach R. Magnification $\times 180$.

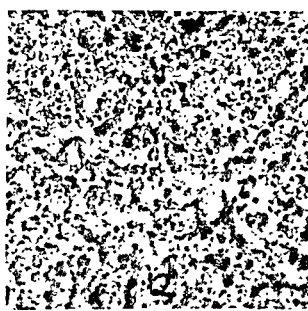
Fig. 11. Photomicrograph of a biopsy of the liver of the same dog used in figure 10 and showing an increase in the severity of the liver damage following the feeding of 100 grams of fresh beef brain daily for one month. Stain Sharlach R. Magnification $\times 180$.

Fig. 12. Photomicrograph of a biopsy of the liver of a depancreatized dog showing moderate fatty infiltration. Stain Sharlach R. Magnification $\times 235$.

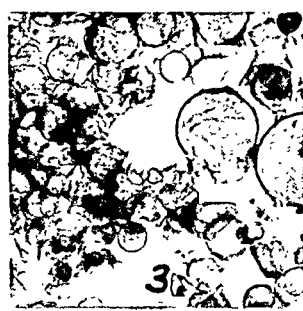
Fig. 13. Photomicrograph of a biopsy of the liver of the same dog used in figure 12 and showing an increase in fatty infiltration after feeding 100 grams of raw beef brain daily for one month. Stain Sharlach R. Magnification $\times 235$.



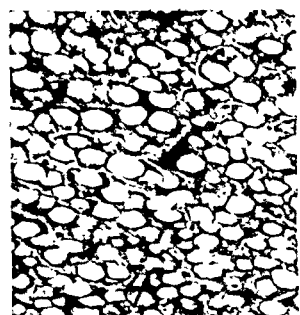
1



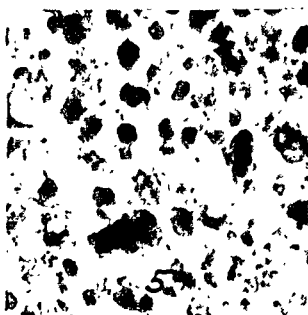
2



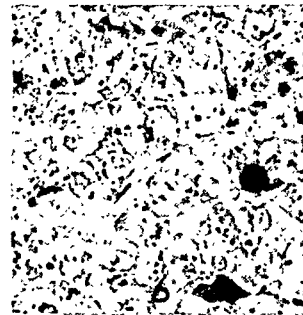
3



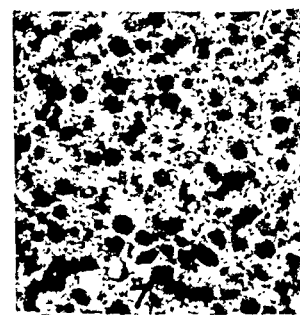
4



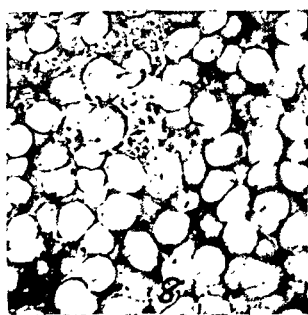
5



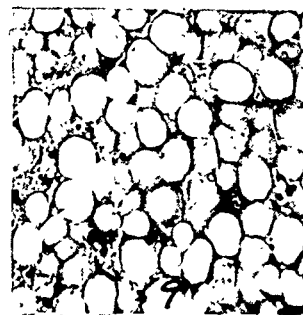
6



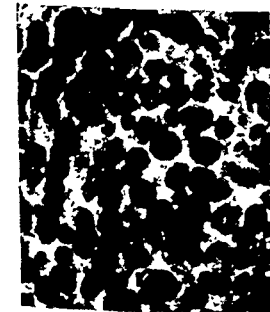
7



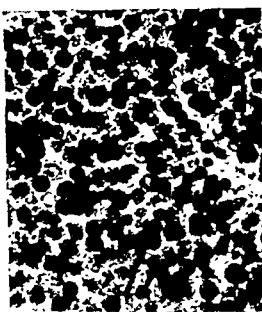
8



9



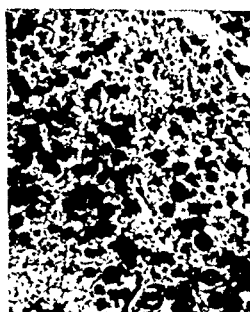
10



11



12



13

Figs. 1 to 13

400 cc. of whole milk. Insulin was administered twice daily and an attempt was made to adjust the dose so as to permit of only a moderate glycosuria. From 20 to 30 units daily were usually required. Fresh activated pancreatic juice usually obtained the same day from pancreatic fistula dogs was fed or given by stomach tube when not taken voluntarily. The amounts given varied between 100 and 1,000 cc. per day. Eight of the animals died during the course of the experiment, the length of survival after the pancreatectomy being 24, 41, 45, 56, 65, 68, 75 and 142 days respectively. In each case at autopsy an extreme degree of fatty degeneration and infiltration of the liver was found. The ninth animal also developed a fatty liver as revealed by biopsy after two months of pancreatic juice administration, and was then used for other studies. Only one of these animals displayed jaundice as a symptom of the liver damage. For the most part the symptoms in the order of their appearance were as follows: a decreased excretion of sugar in spite of constant food intake and insulin administration, increased sensitivity to insulin so that a dose previously well tolerated now caused convulsions, loss of appetite, loss of weight, apathy and muscular weakness. Microscopical sections showing the extensive liver changes in two representative animals appear in figures 3 and 4.

d. *The amount of fresh raw pancreas required to relieve and prevent fatty changes in the liver of the depancreatized dog.* Pancreas contains both lecithin and choline and a further study was now made to determine if these substances could account for the pancreas effect. As mentioned above it has been demonstrated by many workers that the addition of 100 grams of raw pancreas daily to the diet of the depancreatized dog of average size is sufficient when combined with insulin in adequate doses to permit survival in good condition for several years. It seemed important to determine if perhaps a smaller amount of fresh pancreas might suffice, and to secure such data the following experiment was done.

Dog 7. Male. Weight 7.6 kgm. Pancreatectomy 9-19-35. For the first month this animal received 400 grams of meat, 100 grams of bread, 400 cc. of whole milk, 400 cc. of pancreatic juice and 20 units of insulin daily. On 10-18-35 a biopsy of the liver was taken which showed a moderate degree of fatty infiltration (fig. 5). He was then given 25 grams of fresh raw beef pancreas daily in addition to the former diet except that the pancreatic juice was discontinued. A second biopsy of the liver taken on 11-22-35 showed marked improvement (fig. 6). On 12-3-35 the amount of raw pancreas fed was reduced to 10 grams per day, and a third biopsy taken 12-26-35 showed a return of the fatty change in the liver (fig. 7). It would thus appear that for a depancreatized dog weighing about 7 kgm. on a diet such as the standard one used in our experiments, 25 grams of fresh raw beef pancreas is enough to relieve the fatty infiltration of the liver but that 10 grams daily is too small a dose. The amount of choline in the rat pancreas was found by Fletcher, Best and Solandt (1935) to be in the neighborhood of 232 mgm. per 100 grams of pancreas. The amounts of choline, however, which MacLean and Best (1935) found to be effective in preventing or

relieving the fatty changes in the liver of the depancreatized dog varied between 1.5 and 2.25 grams per day. The effective dose of choline was found to be somewhat proportional to the amount of fat in the diet. It does not appear that Best and his associates have made a special effort to determine the minimum effective dose of choline for the depancreatized dog, and since this may vary depending on the diet it seemed desirable to us to determine the minimum dose required under the conditions of our experiments and to compare that with the amount that could possibly be present in an effective dose of raw pancreas.

e. *The amount of choline required to prevent or relieve fatty changes in the liver of the depancreatized dog.* Experiments were performed on four depancreatized dogs kept under standard conditions of diet and insulin administration and given respectively 200, 500, 700 and 1,000 mgm. of choline chloride by mouth per day. The data are summarized in the following protocols:

Dog 9. Male. Weight 12.6 kgm. Pancreatectomy 10-17-35. On 11-12-35 a biopsy of the liver was made and this showed a marked degree of fatty change. Two hundred milligrams of choline chloride were then given daily by mouth in addition to the standard diet. A second biopsy of the liver was made on 12-10-35, and this showed an even more extensive fatty infiltration than the first.

Dog 10. Male. Weight 14.5 kgm. Pancreatectomy 12-11-35. Biopsy on 12-30-35 showed a moderately fatty liver. Five hundred milligrams of choline chloride was then given for 9 days, but without clinical improvement. One gram of choline was given on the 10th day and 2.5 grams on the 11th day, but the animal became progressively worse and died on 1-12-36. Autopsy disclosed an extreme degree of fatty change in the liver.

Dog 11. Male. Weight 12.2 kgm. Pancreatectomy 1-17-36. Diet, 300 grams lean beef, 75 grams bread, 400 cc. of whole milk, and 20 units of insulin daily. Biopsy on 2-18-36 disclosed a moderate fatty infiltration of liver (fig. 8). Seven hundred milligrams of choline chloride was then given orally for 26 days. A second biopsy on 3-16-36 disclosed a more severe degree of fatty change in the liver (fig. 9).

Dog 12. Male. Weight 9.4 kgm. Pancreatectomy 12-12-35. Standard diet plus 10 units of insulin daily. Biopsy on 12-30-35 disclosed a slight fatty infiltration of the liver. One gram of choline chloride was then given orally for 14 days at which time the animal developed pneumonia and died. At post-mortem examination microscopic sections showed definite regeneration of the liver.

The results of this experiment indicate that whereas 1.0 gram of choline chloride daily was sufficient to improve the condition of the liver of the depancreatized dog, smaller doses such as 200, 500 or even 700 mgm. per day were entirely ineffective. Thus while we were able to secure a definite effect with smaller doses of choline than hitherto reported, the minimum amount required was still approximately 15 times as much as is calculated to be present in 25 grams of fresh pancreas, an effective dose of this substance.

f. *The effect of the oral administration of fresh beef brain on the fatty changes in the livers of depancreatized dogs.* Lecithin and choline are present not only in pancreas but in even greater amounts in certain other tissues.

Thus Fletcher, Best, and Solandt (1935) found the brain of the rat to contain approximately 325 mgm. of choline per 100 grams of tissue. Since it was clear that the pancreatic enzymes played no rôle in the beneficial effect of pancreas feeding, experiments were done to determine the effect of the oral administration of comparable amounts of fresh beef brain.

Dog 8, a male, weighing 12 kgm. was depancreatized 12-4-35 and placed on the standard diet and 15 units of insulin daily. A biopsy taken 22 days later on 12-26-35 disclosed a definite fatty infiltration of the liver (fig. 10). One hundred grams of fresh raw beef brain were added to the diet from 12-31-35 to 1-30-36 when a second biopsy was taken. This showed a much more marked fatty degeneration and infiltration of the liver (fig. 11). A similar result was obtained with dog 9 that had been used before in an experiment with choline and with pancreas. This animal, a male weighing 12.6 kgm., was depancreatized 10-17-35, but as a result of treatment was in good condition on 2-3-36 when a biopsy of the liver disclosed only moderate fatty infiltration (fig. 12). One hundred grams of fresh raw beef brain were added to the diet from 2-7-36 to 3-3-36 when another biopsy of the liver were taken. This showed a marked increase in the extent of the liver damage (fig. 13).

g. *The effect of the oral administration of fresh beef liver on the fatty changes in the liver of the depancreatized dog.* The negative results obtained by the feeding of fresh brain tissue suggested that the effect obtained with fresh pancreas was specific. To check this impression further, a trial was now made with fresh beef liver. A depancreatized dog that had developed a fatty liver 27 days after pancreatectomy and had been relieved by pancreas administration was selected for the experiment. A preliminary biopsy of the liver was taken and this revealed definite fatty changes. The animal weighed 11.0 kgm. and on the standard diet and 20 units of insulin daily excreted an average of about 18 grams of sugar in 24 hours. One hundred grams of fresh raw beef liver were then administered daily in addition to the usual diet and insulin. After 12 days of liver feeding the sugar excretion decreased and the insulin dosage was reduced to 15 units. Twenty-four days after the beginning of liver feeding symptoms of liver damage became pronounced and a biopsy revealed a marked increase in the extent of fatty infiltration and degeneration in the liver. During the last 12 days of the experiment the average daily sugar excretion was less than 1 gram although the animal consumed all of his food and received only 15 units of insulin per day.

DISCUSSION. The results obtained in the experiments described above indicate very definitely that the absence of pancreatic juice from the intestine is not the cause of the fatty changes in the liver of the depancreatized dog. The slight degree of fatty infiltration of the liver observed in three of the pancreatic fistula dogs was by no means comparable to the extensive changes seen after pancreatectomy. Furthermore, the fact that this slight fatty infiltration occurred only in the three fistula animals which developed severe infections, whereas the other four had normal livers

suggests that the infection rather than the loss of the pancreatic secretion is the responsible factor. It is probable that a similar explanation may account for the liver changes observed by Berg and Zucker in the pancreatic fistula dogs prepared by cannulating the ducts.

The changes in the livers of two of the animals in which the pancreatic ducts had been ligated were so slight as compared with those after pancreatectomy that we consider the effect negative. The liver of the third animal was normal. These observations suggest that it may not be the loss of acinar tissue which causes the fatty changes in the liver after pancreatectomy, but that this is due to a deficiency in islet function. A definite statement cannot be made since although the great bulk of the acinar tissue of the pancreas in these cases was degenerated, small remnants of apparently normal cells were still present in the periphery of many acini.

The depancreatized dogs given pancreatic juice as a supplement to insulin treatment lived no longer than control animals on insulin treatment alone. Furthermore, the degree of fatty degeneration and infiltration of the liver and the rapidity of its onset after pancreatectomy were either not affected by the administration of the juice or the condition was aggravated. The secretion was given daily in amounts equal to those normally secreted and with only short intermissions for the entire post-operative period. The digestion and absorption of food in the intestines was markedly improved, as evidenced by the increased sugar excretion and the decrease in the bulk of the feces, but if anything, the accumulation of fat in the liver was hastened rather than retarded. It is thus very evident that the beneficial effect of raw pancreas is not due to the pancreatic enzymes it contains or to an improvement in the digestion and absorption of fats occasioned by the presence of those enzymes.

While the observations of Best and his associates that choline will relieve the fatty degeneration of the liver of the depancreatized dog have been confirmed, the amount required was so great that it does not seem possible to account for the pancreas effect on this basis. We were unable to secure a favorable response with less than 1.0 gram of choline chloride daily, whereas the amount of choline in a minimum adequate dose of fresh pancreas (25 grams) we have estimated to be about 60 mgm. The fact that the administration of raw brain or liver (100 grams daily) was unable to prevent or relieve the characteristic fatty changes in the liver is significant since brain contains somewhat more lecithin and choline than is found in pancreas. While it is altogether probable that these substances play an exceedingly important rôle in the transport and metabolism of fat, we believe that the evidence presented is more in harmony with the view that there is some other specific substance in pancreas, whose absence leads to the liver changes described, and whose presence in the fresh pancreas is chiefly responsible for its beneficial effect when fed to depancreatized animals.

CONCLUSIONS

1. The fatty degeneration and infiltration of the liver, which occurs in depancreatized dogs treated with insulin, is not due to the absence of pancreatic juice from the intestines since:

a. It does not occur in dogs provided with total pancreatic fistulae.

b. It does not develop in dogs following ligation of all pancreatic ducts and degeneration of pancreatic parenchyma.

2. The beneficial effect of raw pancreas feeding after pancreatectomy is not due to the pancreatic enzymes since the administration of fresh pancreatic juice has no such beneficial effect.

3. Choline and lecithin are probably not the substances in pancreas which are responsible for its effect in prolonging the survival of depancreatized dogs treated with insulin and in preventing the fatty changes in the liver, since:

a. The minimum effective dose of choline in relieving the fatty changes in the liver of the depancreatized dog is many times greater than the amount present in an effective dose of pancreas.

b. Fresh raw brain has no such beneficial effect, although it contains more lecithin and choline than pancreas.

4. The beneficial effect of raw pancreas feeding after pancreatectomy is probably due to some specific substance in pancreas since equivalent amounts of liver or brain were ineffective in this respect.

REFERENCES

- ALLAN, F. N., J. J. BOWIE, J. J. R. MACLEOD AND W. L. ROBINSON. *Brit. J. Exper. Path.* **5**: 75, 1924.
- BERG, B. N. AND T. E. ZUCKER. *Proc. Soc. Exper. Biol. and Med.* **29**: 68, 1931.
- HERSHEY, J. M. *This Journal* **93**: 657, 1930.
- HERSHEY, J. M. AND S. SOSKIN. *This Journal* **98**: 74, 1931.
- BEST, C. H. AND J. M. HERSHEY. *J. Physiol.* **75**: 49, 1932.
- BEST, C. H. AND M. E. HUNTSMAN. *J. Physiol.* **75**: 405, 1932.
- BEST, C. H., G. C. FERGUSON AND J. M. HERSHEY. *J. Physiol.* **79**: 94, 1933.
- MACLEAN, D. L. AND C. H. BEST. *Brit. J. Exper. Path.* **15**: 193, 1934.
- BANTING, F. G. AND C. H. BEST. *J. Lab. and Clin. Med.* **7**: 265, 1922.
- FISHER, N. F. *This Journal* **67**: 634, 1924.
- RALLI, E., G. FLAUN AND R. J. BANTA. *This Journal* **110**: 545, 1935.
- HARMS, H. P., J. V. PROHASKA AND L. R. DRAGSTEDT. *This Journal* **117**: 160, 1936.
- FLETCHER, J. P., C. H. BEST AND O. M. SOLANDT. *Biochem. J.* **29**: 10, 1935.

OBSERVATIONS ON A SUBSTANCE IN PANCREAS (A FAT METABOLIZING HORMONE) WHICH PERMITS SURVIVAL AND PREVENTS LIVER CHANGES IN DEPANCREATIZED DOGS¹

LESTER R. DRAGSTEDT, JOHN VAN PROHASKA AND HERMAN P. HARMS

From the Department of Surgery of The University of Chicago

Received for publication June 6, 1936

The early report of Fisher (1924), Allan, Bowie, Macleod, and Robinson (1924), and the subsequent extensive studies of Best and his associates (1932, 1933, 1934, 1935) demonstrated quite conclusively that the completely depancreatized dog would not survive more than a few months even though adequately treated with insulin. The most prominent abnormality seen at autopsy was an extreme degree of fatty degeneration and infiltration in the liver. These changes in the liver could be prevented and life indefinitely prolonged by the addition of adequate amounts of raw pancreas, lecithin, or choline to the diet. An exception to these statements is found in the report of Chaikoff (1935) that he has maintained two depancreatized dogs alive and in good condition for over four years by means of insulin but without the administration of pancreas, lecithin, or choline. These probably represent exceptional instances. Furthermore, both animals were still living at the time of the report so that verification of the completeness of the pancreatectomy is not yet available. In the present studies we have depancreatized 45 dogs and all save one developed the characteristic changes in the liver as verified by biopsy or post-mortem examination. Eight of these animals received no supplement to the diet, which was effective in relieving the fatty changes in the liver, and all died very promptly. In the great majority of cases the animals remained in good condition for three to four weeks after the pancreatectomy, excreting considerable amounts of sugar on a diet of 400 grams of meat, 400 cc. of whole milk, and 100 grams of bread with 20 to 30 units of insulin daily. Then the sugar excretion began to diminish, the insulin requirement to decrease, and the animals became apathetic and more and more indifferent to food. Jaundice was rarely observed. Biopsy of the liver taken during the presence of these symptoms invariably revealed an extensive degree of fatty change. We were soon convinced that animals manifesting these symptoms would shortly

¹ This work has been aided by a grant from the Douglas Smith Foundation for Medical Research of The University of Chicago.

die unless some effective supplement was added to the diet. Only one of the 45 animals failed to develop a fatty liver, but this one died 72 days after the pancreatectomy and no significant changes other than the pancreatectomy were found at post-mortem examination.

The findings described in the preceding paper, indicating that the beneficial effect of raw pancreas in preventing and relieving the fatty changes in the liver of the depancreatized dog could not be accounted for on the basis of its lecithin or choline content or by the presence of the pancreatic enzymes, suggested that the effect must be due to some other substance. An attempt was made to secure such a substance in various types of pancreatic extracts. Fractionation of the pancreas was difficult and time consuming because of the nature of the criteria involved. In every instance the activity of a fraction was tested by its ability to relieve already established fatty degeneration and infiltration in the liver of the depancreatized dog. To accomplish this a number of dogs were carefully depancreatized, placed on our standard diet of 400 grams of meat, 400 cc. of whole milk, and 100 grams of bread, and given sufficient insulin daily to permit of only a moderate glycosuria. After a period of four weeks symptoms suggestive of fatty liver usually appeared and a control biopsy of the liver was taken. If this showed definite fatty infiltration the animal was given one of the pancreas extracts mixed with the food. Usually an amount of extract obtained from 100 grams of fresh pancreas was employed and this dose was given daily for a period of 3 to 6 weeks and sometimes longer. A beneficial effect was occasionally manifested in a few days where the fatty infiltration was very marked and the condition of the animal poor; this might be delayed for a week or longer. The symptoms of such improvement were usually increase in appetite, increase in sugar excretion, and renewed activity. In every instance a second biopsy was taken after 3 to 4 weeks and the conclusion as to the activity of the extract tested was based largely on a comparison of the microscopic appearance of the two biopsy specimens. This method proved very reliable since the fatty changes in the liver were found to be practically uniform throughout and improvement following the administration of active preparations equally diffuse and widespread.

EXPERIMENTAL PROCEDURE. Fresh beef or calf pancreas, received usually in a partially frozen state, was stripped of adherent fat, finely minced, and mixed with about two volumes of 95 per cent ethyl alcohol. The mixture was stirred frequently and allowed to extract for five to six hours. The alcohol was then filtered off and the residue treated three more times in similar fashion with 95 per cent alcohol. The alcoholic filtrates were then combined and evaporated in shallow pans at room temperature to a thick brown paste. This was then extracted from two to five times with several volumes of sulphuric ether. The residue was

found to be soluble in 5 per cent NaCl solution and in water. This portion was called the "fat-free alcohol extract" and from 1.8 to 2.5 grams of the dried product was obtained from 100 grams of fresh pancreas. The residue from the original alcohol extractions was then dried and extracted several times (2 to 5) with ether. The various ether filtrates from this extraction and from the extraction of the alcohol extract were combined and evaporated. This residue, which contained practically all of the lipids of the original pancreas, was called the "ether extract." From 10 to 14 grams of this dried ether extract were obtained from 100 grams of fresh pancreas. The residue of pancreas remaining after both alcohol and ether extraction was called the "pancreatic residue." This residue was extracted with 5 per cent NaCl solution, the extract filtered off and labeled "salt solution extract of pancreas residue." In many instances the effect of various extracts was tested alternately on the same animal so as to secure the advantage of comparison and for purposes of control. The results are indicated in the following representative experiments.

Dog 7. Male. Weight 7.6 kgm. Pancreatectomy 9-19-35. Biopsy 10-18-35 showed a fatty liver; 25 grams of raw pancreas were then added to the diet and a second biopsy 11-22-35 disclosed an almost normal liver. The dose of pancreas was then reduced to 10 grams and a third biopsy 12-26-35 indicated a return of fat in the liver (fig. 1). Fourteen grams of "ether extract" of pancreas (the amount obtained from 100 grams of fresh gland) were then fed instead of the pancreas for a period of 19 days and a fourth biopsy 1-14-36 showed a marked increase in the degree of fatty change in the liver (fig. 2). The animal was then given 1.5 grams of the "fat-free alcohol extract" (the amount obtained from 100 grams of fresh gland) and a fifth biopsy 2-18-36 after 34 days administration showed a definite improvement in the appearance of the liver (fig. 3).

Dog 9. Male. Weight 12.6 kgm. Pancreatectomy 10-17-35. This animal developed a fatty liver within one month and this was not relieved but became more marked during the second month while it was receiving 200 mgm. of choline daily as supplement. Biopsy taken on 12-10-35 revealed a very fatty liver (fig. 4). The "fat-free alcohol extract" was then given instead of the choline and a biopsy taken 20 days later 12-30-35 showed some improvement in the liver. The administration of the extract was continued and the condition of the animal improved markedly. Another biopsy taken 2-3-36 showed almost complete recovery (fig. 5). This animal later developed a fatty liver again while given raw brain as a supplement.

Dog 10. Weight 8.6 kgm. Pancreatectomy 11-11-35. A biopsy taken 12-6-35 showed fatty infiltration in the liver (fig. 6). The animal was then fed "pancreatic residue" (amount corresponding to 100 grams of original pancreas) as a supplement and a second biopsy taken 1-7-36 showed definite improvement (fig. 7). The "fat-free alcohol" extract was then given for 27 days and a third biopsy 2-3-36 showed still more recovery. Both the "fat-free alcohol" extract and "salt solution extract of pancreas residue" were then given for 30 days and a fourth biopsy taken 3-4-36 disclosed further improvement (fig. 8).

Dog 12. Male. Weight 8.8 kgm. Pancreatectomy 12-31-35. A biopsy of the liver taken 1-17-36 showed definite fatty infiltration of the liver (fig. 9). "Salt solution extract of pancreas residue" in an amount corresponding to 125 grams of fresh pancreas was then fed for one month and a second biopsy taken 2-18-36 disclosed almost complete regeneration of the liver (fig. 10).

Dog 13. Weight 916 kgm. Hypophysectomy 11-19-35. On 12-3-35 the general condition was good and the blood sugar was found to be 67 mgm. per 100 cc. Pancreatectomy was done on 12-3-35. The animal was then placed on the standard diet but given no insulin. The sugar excretion at first ranged between 5 and 12 grams per day but gradually decreased and after 3 weeks the urine became practically sugar free. A biopsy of the liver taken 1-7-35, 5 weeks after the pancreatectomy, revealed marked fatty changes (fig. 11). One and five-tenths gram of the "fat-free alcohol extract" was then given daily for about 6 weeks and a second biopsy on 2-19-36 showed definite improvement in the liver (fig. 12). During the last 3 weeks of this period the sugar excretion increased to an average of 28 grams per day. No insulin was administered at any time.

Observations were made on nine additional dogs besides those described in the protocols. The results were strikingly uniform throughout. The "ether extract" of pancreas containing the lipid fraction was in no case found to be active but rather seemed to increase the rate of fat deposition

Fig. 1. Photomicrograph showing a moderate degree of fatty infiltration in the liver of a depancreatized dog. Stain Sharlach R.

Fig. 2. Photomicrograph of a biopsy of the liver of the same animal as in figure 1 and showing a definite increase in the degree of fatty change after the administration of the "ether extract" of pancreas for 19 days. Stain hematoxylin and eosin.

Fig. 3. Photomicrograph of a biopsy of the liver of the same animal as in figures 1 and 2 and showing a marked improvement in the liver after the administration of the "fat-free alcohol extract" of pancreas for 34 days. Stain hematoxylin and eosin.

Fig. 4. Photomicrograph of a biopsy of the liver of a depancreatized dog showing a marked degree of fatty infiltration. Stain Sharlach R.

Fig. 5. Photomicrograph of a biopsy of the liver of the same animal as in figure 4 and showing the disappearance of the fat after the administration of the "fat-free alcohol extract" for 53 days. Stain Sharlach R.

Fig. 6. Photomicrograph of a biopsy of the liver of a depancreatized dog showing a moderate degree of fatty change. Stain Sharlach R.

Fig. 7. Photomicrograph of a biopsy of the liver of the same animal as in figure 6 and showing some improvement produced by the administration of "pancreatic residue" for 30 days. Stain Sharlach R.

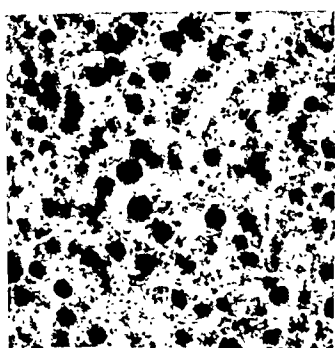
Fig. 8. Photomicrograph of a biopsy of the liver of the same animal as in figures 6 and 7 and showing complete recovery after the administration of "fat-free alcohol extract" and "salt solution extract of pancreas residue" for 57 days. Stain hematoxylin and eosin.

Fig. 9. Photomicrograph of a biopsy of the liver of a depancreatized dog showing marked fatty infiltration. Stain Sharlach R.

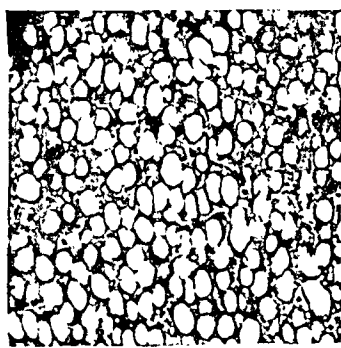
Fig. 10. Photomicrograph of a biopsy of the liver of the same animal as in figure 9 and showing the disappearance of fat after the administration of the "salt solution extract of pancreas residue" for one month. Stain Sharlach R.

Fig. 11. Photomicrograph of a biopsy of the liver of a hypophysectomized-depancreatized dog showing extreme fatty changes. Stain Sharlach R. The fat droplets have not stained deeply.

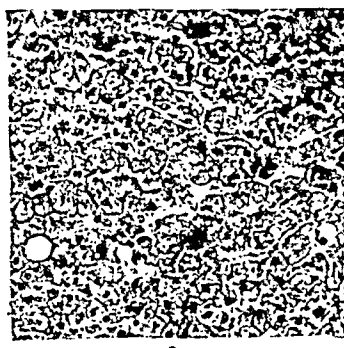
Fig. 12. Photomicrograph of a biopsy of the liver of the same animal as in figure 11 and showing the improvement produced by the administration of the "fat-free alcohol extract" of pancreas for six weeks. Stain Sharlach R.



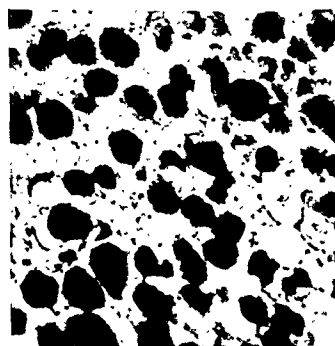
1



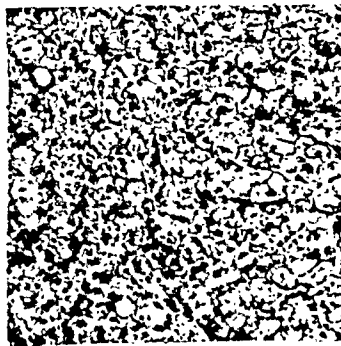
2



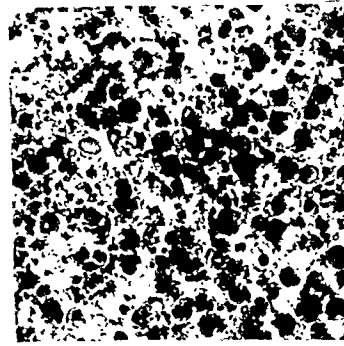
3



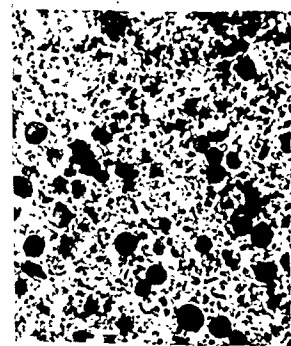
4



5



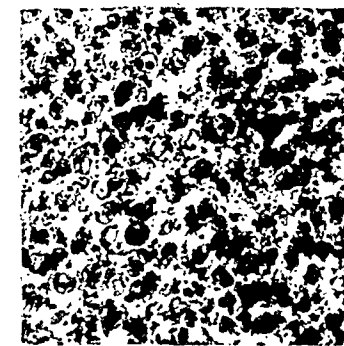
6



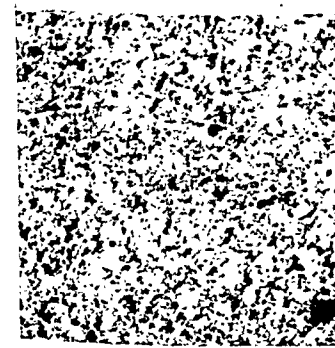
7



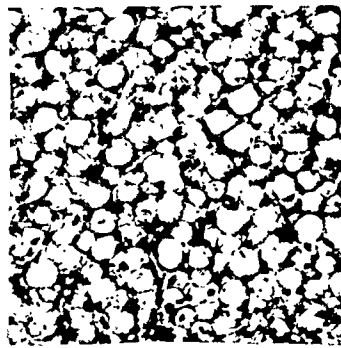
8



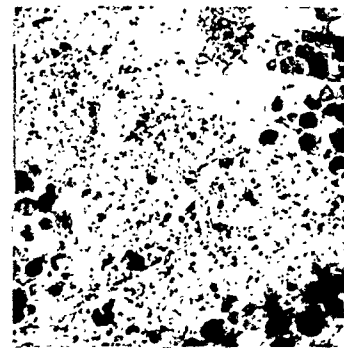
9



10



11



12

Figs. 1 to 12

in the liver. The "fat-free alcohol extract" of pancreas was in each case found to contain the active principle and to be effective in relieving already established fatty liver in depancreatized dogs when given in a daily dose of from 1.0 to 1.5 gram of the dried substance. This was somewhat less than the amount derived from 100 grams of fresh gland. The "pancreatic residue" and the "salt solution extract of the pancreatic residue" were each found to contain the active principle when the original alcohol extractions were carried out with 95 per cent alcohol but in greatly reduced amount when 60 per cent alcohol was used. Apparently the lower concentration of alcohol was a better solvent and removed most of the substance from the pancreas.

Observations were made on one additional hypophysectomized-depancreatized dog besides the one mentioned in the protocols. Neither of these animals received insulin at any time. Both developed the characteristic changes in the liver just as rapidly as the depancreatized dogs in our series and they responded quite as well when given the active extracts.

The facts that have appeared as a result of this study would seem to warrant the conclusion that there is present in fresh pancreas a specific substance, aside from lecithin or choline, which is effective on oral administration in preventing or relieving the fatty degeneration and infiltration of the liver in depancreatized dogs. This substance is insoluble in ether, but soluble in alcohol, 5 per cent NaCl solution, and in water. The fact that it is not present in pancreatic juice makes it seem probable that it is a hormone which under normal conditions plays a rôle in the transport or further utilization of fat. It is possible that choline may play some important intermediary rôle in this function. Hypophysectomy, which lessens the necessity for insulin in conserving the life of the depancreatized dog, does not compensate for the absence of this fat metabolizing hormone.

After consultation with Carl D. Buck, Professor of Comparative Philology of The University of Chicago, we have chosen the name "lipocaic" for this substance. It is derived from the Greek words "*λίπος*," "fat," and "*καίω*," "I burn." A more general term suggesting that the hormone plays a rôle in the utilization of fat was sought but without success.

CONCLUSIONS

A specific substance has been obtained in alcoholic extracts of beef pancreas, that on oral administration to depancreatized dogs treated with insulin, permits survival and prevents and relieves the fatty degeneration and infiltration of the livers of these animals. This substance, for which the name "lipocaic" is suggested, is believed to be a new hormone that is concerned in some way with the normal transport and utilization of fat.

REFERENCES

- ALLAN, F. N., J. J. BOWIE, J. J. R. MACLEOD AND W. L. ROBINSON. Brit. J. Exper. Path. 5: 75, 1924.
- BEST, C. H., G. C. FERGUSON AND J. M. HERSHEY. J. Physiol. 79: 94, 1933.
- BEST, C. H. AND J. M. HERSHEY. J. Physiol. 75: 49, 1932.
- CHAIKOFF, I. L. Proc. Soc. Exper. Biol. Med. 33: 211, 1935.
- FISHER, N. F. This Journal 67: 634, 1924.
- MACLEAN, D. L. AND C. H. BEST. Brit. J. Exper. Path. 15: 193, 1934.

HISTONE COMBINATIONS OF THE PROTEIN HORMONES

FRITZ BISCHOFF

From the Chemical Laboratory, Santa Barbara Cottage Hospital

Received for publication June 8, 1936

In searching for protein precipitants which might be used clinically to delay resorption of parenterally administered protein hormones, the histones, being well known as protein precipitants, presented themselves as more or less obvious possibilities. Trial in experimental animals of the insoluble thymus histone combination of insulin and of the pituitary gonadotropic preparation produced results entirely unexpected, differing from those previously obtained in this laboratory with the zinc, iron, and tannic acid combinations of these hormones (1) (2) (3) (4).

Preparation of histone. The thymus histone prepared according to the method of Kossel and Kutscher (5) did not in our hands yield a product completely acid soluble, and it contained a high ash content. The following modifications were introduced. The first ammonia precipitate, after washing with ammonia water, was taken up in 0.9 per cent HCl. Insoluble material was removed by filtration, after which the filtrate was neutralized with ammonia to a pH faintly pink to phenolphthalein. The slight mostly crystalline precipitate which formed was removed by centrifugation. (It analyzed 46 per cent ash.) The filtrate was then made strongly alkaline with ammonia. From this point the procedure of Kossel and Kutscher was again followed. The resulting product was acid soluble, contained 4 per cent ash¹ and 17.0 per cent nitrogen, gave positive biuret and Millon's tests, was precipitated by trichloroacetic but not by nitric acid. A stock solution containing 10 mgm. per cubic centimeter was prepared by solution in dilute HCl. This was sterilized by heating to 70°C. on three successive days.

Insulin combination. From the isoelectric point of insulin to a pH more alkaline than 8.5 the histone precipitates insulin,² and the resulting sus-

¹ Presumably magnesium ammonium phosphate. Negative H₂S group. Not ammonia soluble. Zinc is ruled out. The absence of zinc is of great importance to the interpretation of the results, since small amounts of zinc, peptized by insulin in mildly alkaline solution, precipitate the insulin at neutrality. The negative results with the pituitary gonadotropic preparation, which is very sensitive to the presence of zinc, corroborate the absence of this metal.

² Fortunately these studies were begun before the appearance of the recent statement of Hagedorn, Jensen and Krarup (J.A.M.A. 106: 177, 1936) that insulin does not form an insoluble combination with histone; we doubtless would have taken the Danish workers at their word. These authors, using serum as a solute for solubility

pension, if standardized by intramuscular injection, shows a marked (at least 75 per cent) decrease in activity (table 1). If the suspension is given intravenously the activity approaches very nearly that of the original insulin. By giving massive doses of the histone precipitate intramuscularly it was found possible to produce prolonged hypoglycemia without producing insulin shock (table 2).

The procedures in testing the insulin were the same as those described in earlier publications (2) (4). In the experiments recorded 1.5 mgm.

TABLE 1

Comparison of intramuscular and intravenous injections of insulin-histone in rabbits

INSULIN PREPARATION	DOSE PER KILO	NUMBER OF RABBITS	ROUTE OF INJECTION	BLOOD SUGARS IN MILLIGRAMS PER 100 CC.		
				1 hour	3 hours	5 hours
Insulin.....	1 u	10	Intramuscular	46 \pm 3	51 \pm 5*	101
Histone-insulin.....	1 u	Same	Intramuscular	77 \pm 3	99 \pm 4	108
Insulin.....	1 u	9	Intravenous	50 \pm 4	59 \pm 6	89
Histone-insulin.....	1 u	Same	Intravenous	50 \pm 4	67 \pm 6	98

* Three rabbits in the controls convulsed. The blood sugar data for these rabbits are not incorporated. The effect is therefore even more striking than the data would indicate.

TABLE 2

Influence of massive intramuscular dosage of insulin-histone upon the blood sugar response in rabbits

(One-fourth the dose given as the histone in these experiments, produced insulin convulsions when given as ordinary insulin.)

RABBIT	BLOOD SUGAR IN MILLIGRAMS PER 100 CC.								
	0 hour	1 hour	3 hours	5 hours	8 hours	10 hours	12 hours	16 hours	24 hours
C	125		72		72	80		116	130
K	110	54	40	62	48		62	80	122
N	140	58	48	58	70		76	90	150
B	120	62	38	52	78	83			114
F	104	50	40	66	74	96			106

histone preparation was used for every 10 units of commercial insulin. This was more than double the amount required to completely precipitate the insulin. Experiments using 0.4 and 0.8 mgm. histone per 10 units insulin produced the same results as those recorded.

Prolan experiments. The prolan was prepared from urine of pregnancy by the conventional fractional alcohol precipitation procedure. The powder was completely water and 50 per cent alcohol soluble. The stand-

tests, failed to consider the remarkable peptizing powers of this medium (an error similar to that made some years back by the Harvard group studying lead salt solubility).

ardization was performed upon 22-23 day old rats of both sexes at five dosage levels using 24 rats at each dosage level, and ascertaining ovarian, seminal vesicle, and prostate weights and presence of corpora lutea. The preparation assayed 25 units per mgm. (Unpublished data of M. L. Long.)

Thymus histone-prolan combination. The addition of a solution of thymus histone to the prolan solution at pH 6.0 produced a precipitate, there being, however, no change in the activity of the prolan as measured by the effect upon ovarian, seminal vesicle, or prostate weight. See table 3. The following experiment indicated the potency was in the filtrate. Solutions of 100 mgm. prolan and 105 mgm. thymus histone were allowed to react at pH 6.0. The resulting precipitate was removed by centrifugation and washed twice with water. The combined filtrates were taken to pH 8, and alcohol added to 47 per cent concentration. The resulting precipitate was removed by centrifugation and washed twice with 47 per

TABLE 3
Effect of thymus histone upon prolan

PREPARATION ADMINISTERED	RECOVERY AS MEASURED BY:			Number of rats
	Ovarian weight	Seminal vesicle weight	Prostate weight	
	per cent	per cent	per cent	
Prolan + histone (1:5), pH 6.0.....	100	100	100	8
Prolan + histone (1:1):				
pH 6.0 precipitate.....	5	5	5	5
pH 8.2 precipitate.....	10	10	10	5
Filtrate, alcohol precipitate.....	50	75	50	5

cent alcohol. The combined filtrates were taken to pH 6.0 and alcohol added to 83 per cent concentration. The precipitate was removed by centrifugation. All precipitates were finally dissolved at pH 5.0 for assay. It will be noted (table 3) that only slight activity was manifested by the histone precipitates. Over half the active material was recovered from the filtrate (usual for an alcohol precipitation). The pH 6.0 precipitate was highly colored. The procedure could doubtless be used in the purification of prolan.

Pituitary gonadotropic preparation. The preparation studied was the same described in an earlier publication (6). The aqueous solution formed a precipitate with thymus histone (pH 6.0-8.0). The resulting product showed a slight decrease in activity as measured by ovarian weight (table 4). No activity was found in the histone precipitate. The fraction of activity remaining after histone treatment was in the filtrate. The data given in the table were for the ratio 1:2::histone:pituitary powder. Increase of the amount of thymus histone used to a ratio of 2:1 did not

produce complete inactivation, there being corpora lutea in the ovaries of the dosed animals.

In order to increase the sensitivity of the assay the experiment was repeated, adding Zn before dosage. The results indicated there was some activity in the histone precipitate (between 12 and 25 per cent) (table 4).

DISCUSSION. The results clearly show that the histone does not combine to form an insoluble compound with prolan and in no way influences the physiologic effect of prolan. In the case of the pituitary gonadotropic preparation most of the active material is not in the histone precipitate, but in the filtrate. On the basis of these findings an augmentation in activity would not be expected. A significant decrease in activity was observed when the assay was performed in the ordinary way while complete recovery of the activity was indicated when the preparations were assayed

TABLE 4

Influence of histone upon the pituitary gonadotropic preparation

PREPARATION ADMINISTERED (TOTAL DOSE PER RAT)	OVARIAN WEIGHT	NUMBER OF RATS
8 mgm. pituitary powder.....	29 \pm 2.4	10
8 mgm. pituitary powder + 4 mgm. histone.....	18 \pm 1.6	7
Precipitate of above histone combination.....	13 \pm 0.9	6
Filtrate of above histone combination.....	21 \pm 2.2	6
1 mgm. pituitary powder + 0.6 mgm. Zn.....	34	5
2 mgm. pituitary powder + 1.0 mgm. Zn.....	83	4
4 mgm. pituitary powder + 2.6 mgm. Zn.....	96	5
8 mgm. pituitary powder + 3.2 mgm. Zn.....	126	4
Precipitate + 2.6 mgm. Zn of 8:8 mgm. histone combination.....	67	6
Filtrate + 2.6 mgm. Zn of 8:8 mgm. histone combination.....	157	5

after the addition of Zn ion (1). The histone apparently combines with naturally occurring protein impurities which, in the conventional assay, serve to decrease the liberation of the active material from the tissue depots by themselves adsorbing the active material. The histone would therefore fill the requirements of those who allude to pituitary inhibitors, only in this case the source would be extrapituitary. On the basis of our findings it is unnecessary to require pituitary inhibitors to explain the physiological findings. It is now well established that the physiological activity of the pituitary gonadotropic extract is largely dependent on the rate of liberation from the tissues. So-called pituitary inhibitors are probably substances like histone, which accelerate liberation of the hormone. Their effect is the opposite of that of zinc or tannic acid which delay the liberation.

In the case of insulin, an insoluble combination with the histone exists

over the pH range found in the body tissues. Since the intravenous administration of this combination produces a hypoglycemic curve in no way differing from that of the original insulin, it may be definitely concluded that insulin is not inactivated by combination with the histone. The marked decrease in activity observed when the histone combination is given intramuscularly and assayed in the conventional manner is markedly in contrast to the results obtained with other insoluble insulin combinations such as the tannate, iron, and zinc combinations. The two former produce an augmentation in effect, the latter a prolongation of effect with a more shallow hypoglycemia curve. It is probable that the histone combination is broken up much more slowly than the other combinations mentioned, and the liberation of most of the insulin spread over such a long period that its hypoglycemic effect (giving 1 u per K) is not detectable in the normal animal. This is borne out by the prolonged hypoglycemia without insulin shock produced by increased dosage of the histone combination. These questions can best be answered by administration of the histone-insulin combination to the diabetic animal. Such studies, clinical in nature, will be presented elsewhere.

A brief summary of the clinical experience of Dr. P. A. Gray with our histone preparation is included.

Histone-insulin on clinical trial. To-date insulin-histone has been used in the treatment of human diabetes in 11 cases. In 3 patients, who have a mild form of diabetes, the defect in metabolism has been satisfactorily controlled by one hypodermic injection of insulin-histone per day. In the other cases the new insulin has been used on conjunction with regular insulin. In all instances to-date the new insulin has been found to be an adequate substitute for old or regular insulin. Its action is noticeable 24 hours after a single injection, but not after 48 hours. Clinical manifestations of hypoglycemic shock follow appropriate dosage. No local or systemic reactions have been encountered.

Case H-6 received one injection of insulin-histone (24 units) before breakfast for each of 4 consecutive days. The fasting blood sugar 24 hours later varied from 114 to 142 mg. per cent. A cross-section of one day follows:

TIME	INSULIN—H UNITS	BLOOD SUGAR	REMARKS	DIET
5:30 a.m.	30	106	Hypoglycemia	C-343
5:40 a.m.				P-91
11:00 a.m.		90		F-86
5:00 p.m.		112		
11:00 p.m.		98		
5:00 a.m.		114		

Insulin-histone is active upon hypodermic injection in human subjects as shown by (1) its ability to control human diabetes mellitus either when used alone or in conjunction with regular insulin, and (2) the production of hypoglycemic shock after appropriate dosage. When it is injected daily a "pooling effect" is noticeable after 4 to 5 days, necessitating reduction in the total daily unitage. The clinical effect of insulin-histone upon a case showing insulin resistance has been striking and will be reported elsewhere.

We are indebted to Miss M. Louisa Long and Mr. Russel Spicer for assistance in the biologic standardization and to Dr. P. A. Gray for his summary of his clinical findings.

SUMMARY

1. The thymus histone precipitates insulin on the alkaline side of the insulin isoelectric point. Given intravenously this combination produces a blood sugar response approximating that of the original insulin. Given intramuscularly a marked decreased in activity is indicated when the assay is made in the conventional manner. Prolonged hypoglycemia without shock is produced by larger doses.

2. Material of low activity is separated from an active filtrate, when thymus histone is added to either prolan or pituitary gonadotropic preparations in the pH range 6.0-8.0. Histone by combining with the naturally contaminating proteins, which adsorb the pituitary gonadotropic principle, produces a decrease in physiological activity when the assay is made in the conventional manner. Complete recovery is indicated when zinc salts are added. The mechanism of the so-called pituitary inhibitors is thus explained.

REFERENCES

- (1) MAXWELL, L. C. This Journal **110**: 458, 1934.
- (2) MAXWELL, L. C. AND F. BISCHOFF. This Journal **112**: 172, 1935.
- (3) BISCHOFF, F. This Journal **114**: 483, 1936.
- (4) BISCHOFF, F. This Journal **116**: 239, 1936.
- (5) KOSSEL, A. AND F. KUTSCHER. Ztschr. physiol. chem. **31**: 188, 1900.
- (6) MAXWELL, L. C. AND F. BISCHOFF. J. Biol. Chem. **112**: 215, 1935.

THE AMERICAN JOURNAL OF PHYSIOLOGY

VOL. 117

OCTOBER 1, 1936

No. 2

SKIN POTENTIAL AND IMPEDANCE RESPONSES WITH RECURRING SHOCK STIMULATION

T. W. FORBES

From the New York State Psychiatric Institute and Hospital

Received for publication April 7, 1936

A slow electrical response following stimulation of the organism has been known, since Vigouroux in 1879 called attention to a direct current resis-

ERRATUM

VOLUME 116, No. 3

On page 557, figure 5, the single aberrant value referred to in the text has been obliterated by the printer. The value was obtained by calculation and was 2.78 liters per square meter per minute 24 minutes after the middle of the meal.

cell interior through pores in the membranes.

There is evidence for more than one factor in phase angle measurements from resting skin. Strohl (1930) on the basis of conductivity data on unstimulated skin has suggested that two tissue structures with different phase angle characteristics are necessary to explain reactance-resistance curves for various frequencies. Cole (1932) has pointed out the entrance of a second factor at high frequencies in measurements of phase angle by himself and others on frog skin.

The present results indicate two *response* mechanisms in a given skin area which may show functional independence.

THE AMERICAN JOURNAL OF PHYSIOLOGY

VOL. 117

OCTOBER 1, 1936

No. 2

SKIN POTENTIAL AND IMPEDANCE RESPONSES WITH RECURRING SHOCK STIMULATION

T. W. FORBES

From the New York State Psychiatric Institute and Hospital

Received for publication April 7, 1936

A slow electrical response following stimulation of the organism has been known, since Vigouroux in 1879 called attention to a direct current resistance change and Tarchanoff in 1890 reported an endosomatic "action current" response. Over 600 papers have been published to date on this "psychogalvanic phenomenon."

Electrodes have usually been attached to palmar and dorsal surfaces of the hand in humans and ear to footpad in animals. In cats the resistance level rises after sympathectomy and the resistance response cannot be elicited (Richter, 1929; Richter and Shaw, 1930; Schwartz, 1934). Evidence has been advanced for a possible parasympathetic factor in palmar resistance in humans and monkeys (Richter, 1929a), and polyphasic endosomatic potential waveform from palm-dorsum leads has been held to indicate sympathetic-parasympathetic balance (Odegaard, 1930).

On the other hand, polyphasic waveforms have been reported from *single* reacting skin areas (Darrow, 1929; Forbes, 1934), and Gildemeister (1928) has formulated an explanation of polyphasic waveform of the response in terms of a unitary neuro-glandular mechanism in which one potential represents an increase in permeability of sudorific cell membranes and the following one results from an exudation of positive ions from the cell interior through pores in the membranes.

There is evidence for more than one factor in phase angle measurements from resting skin. Strohl (1930) on the basis of conductivity data on unstimulated skin has suggested that two tissue structures with different phase angle characteristics are necessary to explain reactance-resistance curves for various frequencies. Cole (1932) has pointed out the entrance of a second factor at high frequencies in measurements of phase angle by himself and others on frog skin.

The present results indicate two *response* mechanisms in a given skin area which may show functional independence.

METHOD. Electrodes. For endosomatic potential response records electrodes consisted of $1 \times 1\frac{1}{2}$ inch zinc plates with gauze pads soaked in $\frac{1}{10}$ saturated zinc sulphate solution, attached to the palm and the dorsum of the left hand of the subject, and to the arm just above the elbow. The skin at the arm electrode was pierced to eliminate responses from this indifferent area and to effect a connection to the body fluids on the inner surface of the area investigated. An insulated hypodermic needle thrust through the skin of the arm and a wick electrode in the mouth were also used for the same purpose. Electrodes for audiofrequency impedance measurements were 1 cm.² zinc, zinc sulfate on palm and dorsum and insulated needle through the skin of the arm. A larger zinc plate electrode (6 cm. diam.) was also used as the inactive electrode for comparison.

Recording. A Cambridge string galvanometer was used for the majority of the records.

To obtain a practically *pure potential* record a high resistance input vacuum tube amplifier was employed. It could be used either as a single stage balanced D.C. amplifier or as a Matthews (1934) balanced input to a second stage. The effective input resistance of this amplifier was approximately 6 megohms and resistance variations of 100,000 ohms produced no apparent deflection. The balanced pure resistance connection eliminated distortion from the use of condensers and from any applied voltage on the skin from the grid bias. The output from this balanced stage was observed direct on the string galvanometer for accurate recording of waveform but was coupled through 25 microfarad electrolytic condensers to a single stage with a 60 microampere Weston type 600 meter in a balanced output circuit for routine recording. A light aluminum rod pointer attached to this meter recorded in the light beam of the string galvanometer.

This meter recorder showed an initial lag of approximately 0.04 second due to the inertia of the meter, and approximately 0.25 second in reaching a 1 millivolt change of level. However, the recorder did not distort the waveform appreciably as shown when it was used simultaneously and in parallel with the string galvanometer.

Method for A.C. analysis. In order to obtain a further electrical analysis of the response our audiofrequency bridge recording was used. In this method an alternating current (approximately 40 μ A) is passed through the skin which forms one arm of an alternating current Wheatstone bridge. The Wheatstone bridge gives, in usual fashion, the capacitance and resistance of the skin before and after the response. Our bridge is so designed, however, that the unbalance occurring *during* the galvanic skin response is recorded photographically from the figure on a cathode ray tube. This arrangement allows the registration of changes which occur too rapidly to be accurately followed by manual balancing of the bridge.

The amount of the *unbalance* is applied across one pair of deflecting plates of the cathode ray tube while a comparison voltage from a 100 ohm pure resistor (R_7 , fig. 1) is applied across the other pair of plates. In this fashion the engineering technique of measuring phase angle changes on the cathode ray tube¹ by recording current versus voltage is applied to the bridge, allowing higher amplification and greater sensitivity than could be obtained by the use of the method without the bridge.

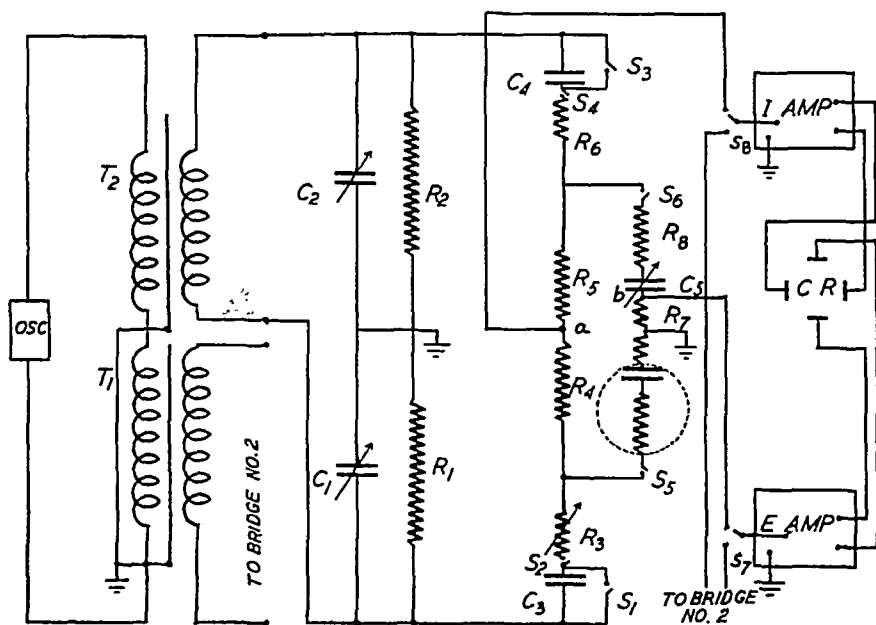


Fig. 1. Audiofrequency bridge and cathode ray circuit. T_1 and T_2 = shielded bridge transformer G. R. Co. type 578A; C_1 , C_2 , R_1 , R_2 = Wagner ground arrangement; C_3 , C_4 = blocking condensers $0.1 \mu\text{F}$ mica and $1000 \mu\text{F}$ variable air; R_3 , R_6 = swamping resistances = 10,000 ohms non-inductive; R_4 , R_5 = 10,000 ohms; R_8 = 100,000 ohm non-inductive decade resistor; C_5 = 0.001 to 1.0 microfarad decade capacitance in parallel with $2,000 \mu\text{F}$ air condenser. X = unknown; CR = 5 in. R. C. A. cathode ray oscillograph. I amp. and E amp. = matched capacitance coupled amplifiers with flat response from 20 to 15,000 cycles. Resistors and decades are all G. R. Co. non-inductive wound. Bridge no. 2 the same as bridge no. 1.

Figure 1 shows one of the two bridges which is an improvement of one previously described and for which operation is identical (Forbes and Landis, 1935).

Under ordinary conditions of resistance and capacitance balance is shown by a straight horizontal line, a resistance variation alone causes a tilting of the figure and a capacitance variation causes it to widen into an ellipse.

¹ I am indebted to Dr. K. S. Cole of the Department of Physiology, College of Physicians and Surgeons, Columbia University, for originally suggesting the use of the cathode ray phase angle measurement.

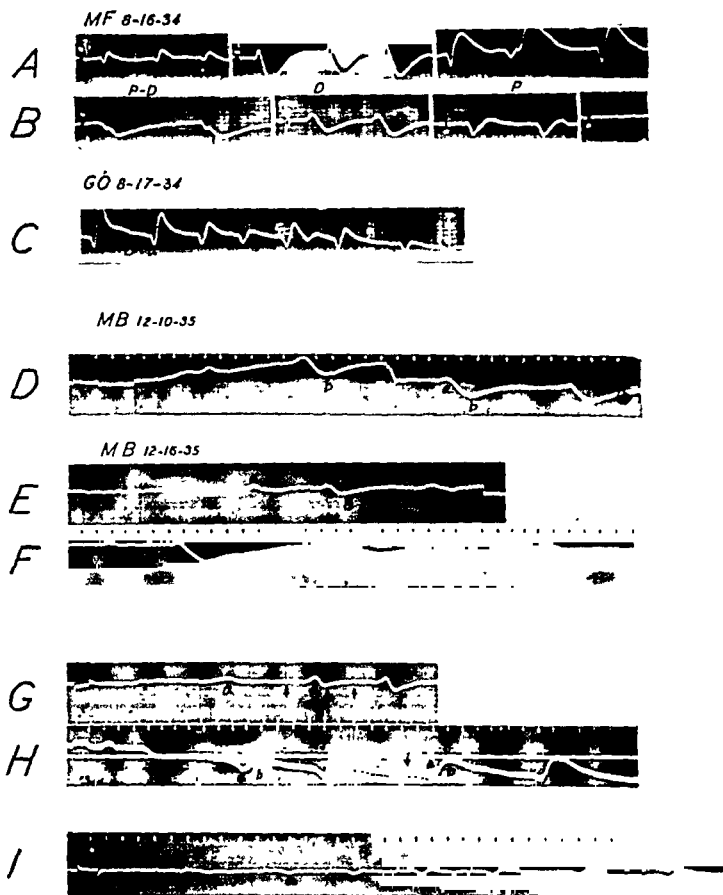


Fig. 2. A, B and C = galvanometer records from single and combined skin areas. Electrodes = zinc plates (6 cm. diam.) with gauze pads and 1/10th saturated zinc sulphate solution. *P* = palm, *D* = dorsum of hand, indifferent electrode on inner forearm, skin pierced. *P-D* = one electrode on palm and one on dorsum. Negative deflection = down for *P*, up for *D*. Original sensitivity = 1 mv. per cm. (standard signal shown). One second time lines retouched. *A* = initial response, *B* = response after 10 or 12 consecutive stimulations. Stimulus artifact can be seen in original records preceding the *a* deflection; *C* = effect of consecutive stimulation.

D, *E* and *F* = comparison of string galvanometer and potential record. Active electrode = $1 \times 1\frac{1}{2}$ inches zinc, zinc sulphate on palm. Inactive electrode = gauze wick in mouth, 1 mv. signal shown. *E* = potential amplifier and string, *D* and *F* = string galvanometer, skin potential balanced, *D* = initial, *F* = later stimulation, arrow = strong shock. Note effect of residual resistance change in *D*.

G, *H* and *I* = wave form with needle electrodes. Active electrode = insulated 25 gauge steel needle above elbow, 1 mv. signal shown. Arrows indicate strong shock stimulus. *G* and *I* = string record, *H* = potential amplifier, *H* and *I* = fall = negative. Note gradual entrance of *b* deflection in *I*.

Simultaneous measurements from two different skin areas were made possible by the use of two bridges, a separate cathode ray figure for each skin area being obtained by switching automatically from bridge to bridge by means of an electronic selector (Hughes, 1936). Interaction between bridges was negligible.

Simultaneous recording of endosomatic potential response with the impedance response was possible by insertion of blocking condensers C_3 and C_4 , which prevented short circuiting of the slow potential response through the transformer T_2 . The potential response is then seen as movement of the whole cathode ray figure up and down. The endosomatic potential variations thus recorded are subject to distortion from C_5 and the unknown C , but serve to indicate relative time relations.

Stimulation. The stimulus used was an electric shock applied through moistened pads to the finger tips of the right hand (recording electrodes on

TABLE 1
Latencies and durations

DEFLECTION	FIGURE	LATENCY	DURATION	AREA
		<i>seconds</i>	<i>seconds</i>	
a	2ABC	0.8-1.0	0.5 -2.0	
a	2D-I	0.6-2.0	0.8 -	
b	2ABC		2.0 -6.0	
b	2GHI		1.0 -2.0	Palm
			1.25-3.0	Dorsum
b	2DEF		1.25-7.0	Palm-dorsum or mouth-palm

left hand). It was found necessary to have the subject administer the shock himself by touching the pads in order to eliminate uncontrolled psychological stimuli (apprehension). A very light movement of the second finger sufficed to administer the shock and such movement in itself was too slight to result in an electrical skin response. The stimulus voltages used were adjusted to give a distinct shock but one felt by the subject to be bearable. The voltage was kept constant for each subject but ranged for different individuals from 90 to 180 volts from B batteries. One-half voltage ("ordinary") and full voltage ("strong") break shocks from a Harvard inductorium with 6 volts on the primary and secondary at setting 2 were also used. The finger was moistened for some two or three minutes before starting stimulation to saturate the skin and to keep the sensory shock as constant as possible (Forbes and Bernstein, 1935). Between stimulus series the fingers were remoistened.

RESULTS. A. *Endosomatic response with plate electrodes.* From 4 adult

human subjects, 2 male and 2 female, a diphasic response was obtainable from palm and from dorsum alone with great regularity. The response from palm and dorsum in series showed a variation in waveform explainable as a summation of the palmar and dorsal responses (c.f. figs. 2A and B). The diphasic waveform has been divided for convenience into an *a* and *b* deflection, as shown in the figure. The latencies and durations were variable (see table 1). The duration of the *b* wave from plate electrodes apparently varied with amplitude.

As a result of repeated stimulation, the *b* deflection gradually diminished until an apparently monophasic form resulted (fig. 2C). The monophasic form is apparently the *a* deflection remaining after the elimination of the *b* deflection. The *b* deflection may start before the *a* deflection is completed. (See apparent shortening of *a*, fig. 2A, P, compared with fig. 2B, P.)

The resultant waveform from two areas in series (P-D records) may be either diphasic, double monophasic, or apparently monophasic (figs. 2A and B, P-D records). Two plate electrodes of the inactive type about one inch apart (skin pierced) showed no response.

B. *Endosomatic response with needle electrodes.* The different waveforms with plate electrodes might still be summation effects if the indifferent area were not effectively eliminated by piercing. This, however, was not the case since similar results were obtained from three individuals using as an indifferent electrode a steel hypodermic needle thrust through the skin of the arm just above the elbow and also by using a wick electrode in the mouth where the absence of sweat glands has been held to eliminate the Tarchanoff effect. Similar records were obtained using in place of the simple string galvanometer, the balanced, high resistance input amplifier with meter or string galvanometer.

No difference in form or latency was noticeable using an indifferent needle electrode through the dorsum as compared to one above the elbow, and interchanging the placement of needle and plate along the axis of the arm did not reverse waveform. The latencies of the *a* and duration of the *b* responses with the needle electrode were again variable. (See table 1, figs. 2G, H and I.)

The use of two plate electrodes allowed distortion of the potential response by the resistance change occurring with it (fig. 2D). The needle electrode by its series capacitance effect selected out the more rapid potential phase (fig. 2G), especially of the *b* deflection. Pure potential records from mouth to palm show the same shortened period (fig. 2E) as compared to string galvanometer records (fig. 2F). It will be noted that this difference cannot be explained on a basis of greater amplitude since the small amplitude responses of figure 3F also show a longer duration. Both string and pure potential records showed a return of the *b* deflection with more intense shock stimulus (arrows in figs. 2G and H).

An impedance change occurred with both *a* and *b* potentials as shown by records on five individuals at 50 and 100 cycles on the audiofrequency

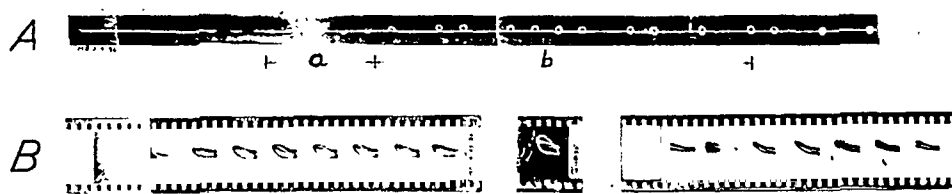


Fig. 3. A. Simultaneous impedance and potential record. Active electrode on left palm, inactive = insulated needle. A. C. Bridge with blocking condensers (C_3 and C_4 , fig. 2) in circuit. Frequency 500 cycles. B = response to break shock on finger of opposite hand. Movie recording, approximately 8 frames per second.

a = negative potential, *b* = positive potential. White zero line drawn in. Unretouched frames appear lighter. Balance = $C = 0.1041 \mu F$, $R = 4660 \Omega$. Total C variation approximately $0.003 \mu F$.

B. Impedance response simultaneously from palm and dorsum. Stimulus = break shock to opposite hand, records with bridge of figure 2. Palm = lower figure, dorsum = upper figure; active electrode = 1 cm.^2 zinc, zinc sulfate; inactive electrode = hypodermic needle above elbow. Simultaneous recording with electronic selector, frequency 100 cycles, continuous current stimuli. Sections are respectively 1st stimulation, residual response after 10 stimulations, and strong shock at 17th response.

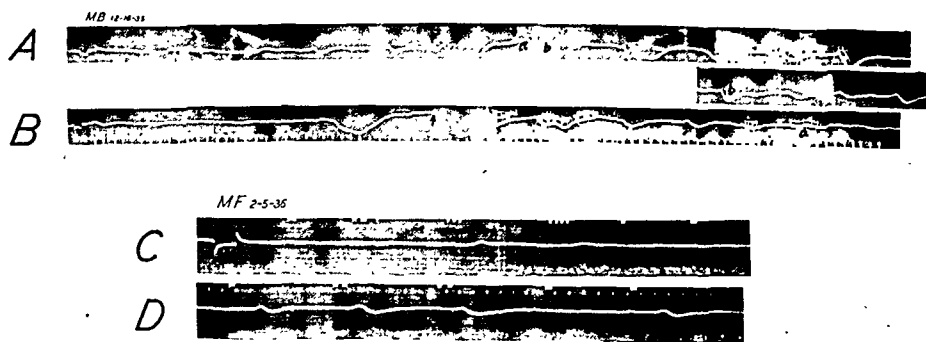


Fig. 4. A and B—effect of apprehension. Active electrode = $1 \times 1\frac{1}{2}$ inch zinc plate on palm; inactive = wick electrode in mouth, string galvanometer recording, successive shock stimuli. A = divided record, heavy shock occurred unintentionally at second arrow, B = similar series after accidental shock has been made impossible. Arrows = heavy shocks.

C and D—summation, palm $1 \times 1\frac{1}{2}$ inch zinc, to needle. Induction break shock indicated by marker, 6 volts, secondary at position 2. C = $\frac{1}{2}$ strength, D = full shock (20,000 Ω potentiometer across secondary). Seconds indicated.

bridge. Figure 3A shows a widening of the cathode ray figure (impedance) beginning during the *a* and a larger change occurring during the *b* potential.

Similar records with the *a* potential only lack the marked impedance response shown in figure 3A with the *b* wave.

Palmar and dorsal skin areas show generally similar waveforms (see fig. 3B) and a similar type of impedance change. Both show impedance (widening) and resistance (tilting) changes but the palm (lower ellipse) shows the greater amplitude. A residual (not completely reversible) impedance drop occurs with repeated stimulation (fig. 3B).

C. Anticipation and summation effects. The *b* potential at times re-entered ahead of the strong stimulation from an at first unrecognized psychological stimulus (fig. 2,I). Figure 4A shows a divided record of a continuous series with two strong shocks in the middle. The second of these was unintentional and produced some apprehension of a repetition. The *b* deflection did not diminish more than shown for a further period of several minutes. Upon rearranging the stimulus set up so as to make impossible any such accidental heavy shock, the *b* deflection diminished rapidly after a strong stimulation. (Cf. fig. 4B with 4A.) This observation has been corroborated with a similar set up intentionally produced with 3 adult subjects.

Both potentials show summation (figs. 4C and D).

DISCUSSION. Our records show, we believe, for the first time a *predictable* potential waveform from the skin of the palm and from the dorsum separately, and indicate that the response consists fundamentally of two opposite and independent potentials from a single skin area. Previous reports of variously diphasic or polyphasic waveforms have been made (Tarchanoff, 1890; Gildemeister, 1923; Darrow, 1926; Ödegaard, 1930, and others). In these studies, however, two reacting areas were used in series and a polyphasic and variable waveform is therefore understandable. A similar technique of using a single reacting area and a non-reacting pierced area has been used without finding a predictable waveform (Darrow, 1929), but apparently a resistance variation similar to those in our figure 3D entered in some of these records to disturb the potential waveform.

It is suggested that each pure potential wave represents liberation of mediator and the slower resistance or impedance response represents the associated permeability change in membranes.

It is probable that the resistance change (permeability) is actually a second slower process and that the difference between figure 3E and F is not merely the *b* potential accentuated by a completely simultaneous resistance variation. Since $D = \frac{E}{R + 4000}$, where *D* is deflection, *E* the somatic potential, 4000 the resistance of the string, *R* the effective skin resistance in the range of 20,000–50,000 ohms, and $\frac{\Delta R}{R}$ is ordinarily less than 10 per cent, and since duration is roughly proportional to amplitude in

this type of record, less than a 10 per cent increase in duration can be thus accounted for.

The electrical response of the skin has been widely held to be a unitary response accompanying activity of the sudorific mechanism. (For summary, see Gildemeister, 1928, and Landis, 1932.) Our results suggest that there are two mechanisms represented and that the *b* potential is connected with a diffuse type of sympathetic activation, while the *a* potential appears non-sympathetic. Evidence for this consists of the independent variation in amplitude of the *a* and *b* potentials, their apparent separate occurrence, and the apparent dependence of the *b* deflection on an intense or an apprehension producing stimulus. The occurrence of summation of action potential has been held to indicate secretion rather than excitation in sympathetic and parasympathetic mechanisms (Rosenblueth, Forbes and Lambert, 1933), and on this basis the occurrence of summation in both *a* and *b* potentials would favor the idea of two separate reacting mechanisms.

The occurrence of a decrease of impedance with either *a* or *b* potentials is evidence that both mechanisms involve changes of tissue permeability.

There is a further suggestion of two mechanisms since both the *a* and *b* potentials show the characteristics of the "quick" potentials reported from the submaxillary gland (Rosenblueth, Forbes and Lambert, 1933). Our *a* potential is similar in latency and duration to the quick potential from single shocks to the chorda obtained by these investigators; while the *b* potential is similar in latency and form to their quick potential with tetanic stimulation of the same nerve. It differs from their slow potential in the greater steepness of the initial rise of the *b* wave as well as a tendency to a shorter duration. The magnitude of the potentials is similar in range (0.5 to 4 or 5 mv.). If both are "quick" potentials we should expect two reacting mechanisms.

The possibility that the *a* and *b* deflections are responses of the *same* mechanisms, the *a* to a single shock and the *b* to a repetitive type of stimulation from ganglia or higher centers does not seem plausible, due to the opposite polarities of the two waves.

The lag in the appearance of moisture in microphotographs (Darrow, 1932) is so great that we can draw no conclusions from the latency reported.

The *a* and *b* potentials show a superficial resemblance to smooth muscle potentials reported by Lambert and Rosenblueth (1935). However, the form is most similar to their potentials I and II, while the latencies and durations are more similar to those of potentials IIIa and IIIb even after allowance for increased conduction time. Further, our potentials *a* and *b* are on the order of millivolts, a much greater magnitude than that reported for the smooth muscle potentials. It does not seem likely, therefore, that either of the two potentials arises from smooth muscle.

The diphasic waveform apparently does not represent transmission of a

wave of activity past the two electrodes, since no reversal of waveform was obtained with reversed positions of needle and plate electrodes.

Evidence of nervous control of sweat glands other than sympathetic has been reported in monkeys (Richter, 1929) and cats (Richter and Shaw, 1930). Vasodilator effects from posterior roots are known (Kuntz, 1934), evidence for vasodilator effects from C fibers in the posterior roots has been reported by Bishop, Heinbecker and O'Leary (1933, 1936), Pinkston, Partington and Rosenblueth (1936). Schwartz (1934) found the intrinsic reflex (resistance) to be definitely sympathetic with a suggestion of a vasodilator as well as a sudorific mechanism, but did not study potentials. It is suggested that our *a* potential may be connected with one of these mechanisms.

I am indebted to Dr. C. Landis for the use of his references on the literature, to Mr. Wm. McKnight and Miss Marjorie Bolles for aid in preparing the manuscript and for technical assistance.

SUMMARY

1. Electrical response potentials were recorded from the skin of the palm and of the dorsum of the hand separately by means of a Cambridge string galvanometer with a high resistance, balanced input amplifier and an alternating current Wheatstone bridge. Simultaneous D.C. and A.C. records were also obtained.

2. The response potentials from each area were similar and consisted of a negative *a* potential followed by a positive *b* potential. The *b* potential apparently overlapped the *a*. The latencies were somewhat variable and ranged from 0.6 to 2.0 seconds for the *a* wave.

3. The *b* potential lessened in amplitude with successive stimulation. The *a* wave was obtainable alone with both weak and strong shocks while the *b* response characteristically entered with strong stimuli when they were such as to cause apprehension or excitement. Both showed summation. Individuals showed differences in the ease of reinstatement of the *b* response.

4. A predictable waveform from the skin has thus been demonstrated by recording pure potentials from a single area. The majority of "potential" waveforms previously reported are probably algebraic summations of the *a* and *b* potentials from two reacting areas, distorted by the resistance response from both areas.

5. The alternating current impedance response did not show, on strong stimulation, any change of form corresponding to the entrance of the *b* potential. An impedance variation was associated with each potential.

6. The sympathetic sudorific mechanism is known to be the source of an electrical potential and resistance response of the skin. The *b* potential shows the diffuse sympathetic character to be expected from sudorific

activation but it is suggested that the *a* potential represents a second, somewhat independent nervous response mechanism. Independence of the two waves, similarity to "quick" glandular potentials found by other workers, and the apparent dependence of the *a* and *b* responses upon different types of stimulating conditions favor this view.

REFERENCES

- BISHOP, G. H. AND P. HEINBECKER. *This Journal* **114**: 179, 1936.
 BISHOP, G. H., P. HEINBECKER AND J. L. O'LEARY. *This Journal* **106**: 647, 1933.
 COLE, K. S. *J. Gen. Physiol.* **15**: 641, 1932.
 DARROW, C. W. *J. Exper. Psychol.* **10**: 197, 1927.
 J. Exper. Psychol. **12**: 267, 1929.
 J. Gen. Psychol. **7**: 261, 1932.
 FORBES, T. W. *Psychol. Bull.* **31**: 698, 1934.
 FORBES, T. W. AND A. L. BERNSTEIN. *J. Gen. Psychol.* **12**: 436, 1935.
 FORBES, T. W. AND C. LANDIS. *J. Gen. Psychol.* **13**: 188, 1935.
 GILDEMEISTER, M. *Pflüger's Arch.* **200**: 278, 1923.
 Hbuch d. norm. u. pathol. Physiol. **8**²: 766, 1928.
 HUGHES, H. K. *Rev. Sci. Instr.* **7**: 89, 1936.
 KUNTZ, A. *The autonomic nervous system.* Lea and Febiger, Philadelphia, 1934, p. 172.
 LANDIS, C. *Psychol. Bull.* **29**: 693, 1932.
 MATTHEWS, B. H. C. *J. Physiol.* **81**: 28, 1934.
 ODEGAARD, O. *Acta Psychiat. et Neurol.* **5**: 55, 1930.
 PINKSTON, J. O., P. F. PARTINGTON AND A. ROSENBLUETH. *This Journal* **115**: 711, 1936.
 RICHTER, C. P. *Bull. Johns Hopkins Hosp.* **45**: 56, 1929.
 This Journal **88**: 596, 1929.
 RICHTER, C. P. AND M. B. SHAW. *Arch. Neurol. and Psychiat.* **24**: 1107, 1930.
 ROSENBLUETH, A. *This Journal* **102**: 12, 1932.
 ROSENBLUETH, A., A. FORBES AND E. LAMBERT. *This Journal* **105**: 508, 1933.
 SCHWARTZ, H. G. *This Journal* **109**: 593, 1934.
 STROHL, A. *Bull. Officiel de la Soc. franc. d'Électrothérapie* **38**: 295, 1930.
 Bull. Off. de la Soc. franc. d'Électrothérapie **38**: 329, 1930.
 TARCHANOFF, J. *Pflüger's Arch.* **46**: 46, 1890.
 VIGOUROUX, R. *Compt. Rend. Soc. Biol.* **31**: 336, 1879.

SURVIVAL OF THE ADRENALECTOMIZED NEPHRECTOMIZED RAT

DWIGHT J. INGLE AND EDWARD C. KENDALL

From the Section on Biochemistry, The Mayo Foundation, Rochester, Minnesota

Received for publication May 14, 1936

It has been proved that the increased excretion of sodium and chloride after adrenalectomy in dogs, cats and rats is of fundamental importance. The loss of electrolyte precedes the terminal syndrome of adrenal insufficiency. Both Loeb (4) and Harrop (1) have explained their experimental results by an hypothesis which assumes that the essential action of cortin is brought about by its effect on the kidney itself. If the action of cortin were confined to its effect on the kidney, the adrenalectomized animal, during its survival after nephrectomy, should not manifest the symptoms of adrenal insufficiency, and the administration of cortin should not benefit such an animal. Three years ago one of us (Ingle) noted that the anesthetized working rat showed a longer survival after nephrectomy than it did after adrenalectomy. After both adrenalectomy and nephrectomy the survival period was shortened to a few hours. Although this result is contrary to the hypothesis in regard to the place of action of cortin, the experiment is not conclusive because of the shock from the operation and the abnormal condition which is produced by the accumulation of toxic products. In the present study the effects of cortin on the survival of the adrenalectomized, nephrectomized animal have been determined.

METHOD. Adult male rats were closely matched, on a basis of age and weight, into groups of three. One animal of each group had only the kidneys removed. A second had both the kidneys and adrenals removed and received subcutaneous injections of cortin every eight hours. A third had both the kidneys and adrenals removed but did not receive any treatment. All operations were performed in a single stage. Aseptic precautions were not taken. Ten groups of animals, which will be termed the "non-work" series, were kept in the usual housing cages with an excess of food and water until death. Ten additional groups of animals ("work" series) were anesthetized with phenobarbital sodium, and immediately following operation the gastrocnemius muscle was stimulated to lift a 100 gram weight three times per second until death. The methods employed have been described previously in detail (2, 3).

RESULTS. In the "non-work" series, the average period of survival of

the nephrectomized animals was 89 hours, with a range of 58 to 130 hours. The adrenalectomized nephrectomized animals treated with cortin showed an average period of survival of 86.7 hours, with a range of 56 to 136 hours. The periods of survivals of the untreated adrenalectomized, nephrectomized animals were much shorter with an average of 19.5 hours and a range of 5 to 45 hours. The amount of urea in the blood of some of the nephrectomized animals at time of death varied from 450 to 974 mgm. per 100 cc.

The effect of work and anesthesia was to shorten the average time of survival to less than that seen in the "non-work" series. Those animals subjected only to nephrectomy had an average period of survival of 52.6 hours with a range of 34 to 65 hours. The adrenalectomized, nephrecto-

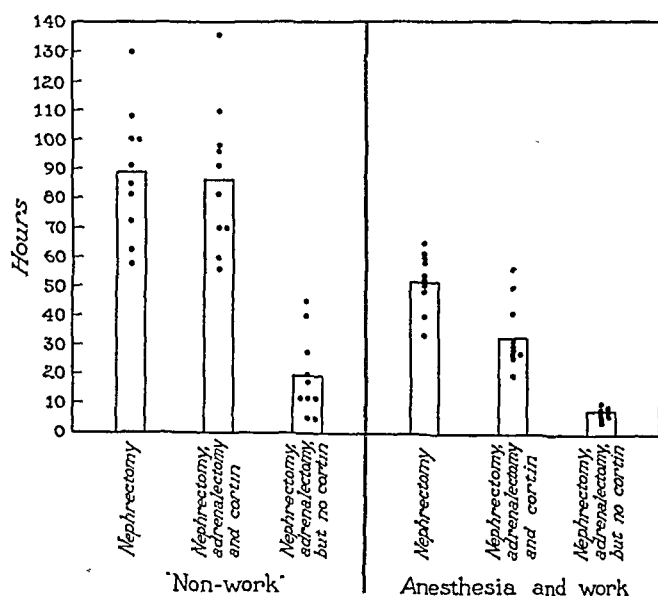


Fig. 1. Effect of cortin on the performance of the adrenalectomized, nephrectomized rat.

mized animals which were treated with cortin had an average period of survival of 33.7 hours, with a range of 20 to 56 hours. The untreated adrenalectomized, nephrectomized animals had an average survival of 7 hours, with a range of 4 to 10 hours. Examination of the total work records in this series showed that the best performance of any untreated, adrenalectomized, nephrectomized rat was still far inferior to the poorest performance among the treated group. The best average performance was shown by those nephrectomized animals whose adrenal glands were left intact. The results are summarized in figure 1.

COMMENT. Removal of the kidneys, which closes the most important avenue for loss of salt from the body, does not prevent death from adrenal

insufficiency. The administration of cortin has a striking effect in prolonging the survival of the adrenalectomized, nephrectomized animal. Adrenalectomized, nephrectomized rats may be conveniently used in the work test (3) to determine the presence of activity in extracts of the adrenal cortex. By this test the presence or absence of activity in an extract can be determined within twelve hours. None of numerous tests have been found in error by subsequent evaluation by other reliable methods. No attempt has been made to standardize this test for quantitative assay.

The periods of survival of the animals of the "non-work" series which were treated with cortin compared favorably with the periods of survival of similar animals which had not undergone adrenalectomy. In the "work" series the survival time of the animals treated with cortin remained less than the survival time of those animals whose adrenals were intact. Under conditions of great stress the inferiority of the animals treated with cortin may only reflect the inability of intermittent injection of the hormone to duplicate the full effect of the continuous secretion by the glands themselves.

It seems probable that the action of cortin is not confined to any single tissue. It will act in the absence of the whole gut, the pituitary body, the thyroparathyroid apparatus, the thymus, the gonads, and after nearly complete removal of the pancreas. Lesions in any part of the hypothalamus do not prevent its beneficial effect in the adrenalectomized animal.

SUMMARY

The periods of survival of nephrectomized rats were compared to periods of survival of adrenalectomized, nephrectomized rats with and without cortin treatment. Ten groups of animals were anesthetized and the gastrocnemius muscle stimulated to contract three times each second until death. A second series of animals was studied under "non-work" conditions. It has been demonstrated that the administration of cortin markedly prolongs the time of survival of adrenalectomized, nephrectomized rats under ordinary living conditions and under conditions which include work and anesthesia.

REFERENCES

- (1) HARROP, G. A., L. J. SOFFER, W. M. NICHOLSON AND M. STRAUSS. *J. Exper. Med.* **61**: 839, 1935.
- (2) HERON, W. T., W. M. HALES AND D. J. INGLE. *This Journal* **110**: 357, 1934.
- (3) INGLE, D. J. *This Journal* **116**: 622, 1936.
- (4) LOEB, R. F., D. W. ATCHLEY AND J. STAHL. *J. A. M. A.* **104**: 2149, 1935.

EFFECT OF EPINEPHRINE ON GLUCOSE EXCRETION IN FASTED DEPANCREATIZED DOGS

W. H. BACHRACH, W. B. BRADLEY AND A. C. IVY

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago

Received for publication May 18, 1936

Probably the strongest evidence in support of gluconeogenesis from fatty acids was that presented by Chaikoff and Weber (1) showing that epinephrine injected into starving depancreatized dogs resulted in the excretion of more glucose than could be accounted for from all sources other than fatty acids. Bollman, Mann and Wilhelmj (2) repeated this work and found that the extra glucose excreted was well within the limit of carbohydrate stores. In fact, by determining muscle glycogen before and after epinephrine administration they found that the extra glucose was equal to the amount of glycogen removed from the muscles during this period. Chambers, Himwich and Kennard (3) also consider muscle glycogen as the source of the extra glucose excreted upon epinephrine administration. Because of the discrepancies in the results reported, the experiment was repeated as originally described by Chaikoff and Weber.

METHOD. Fourteen female dogs were completely depancreatized and placed on a stock diet to which fresh ground pancreas (200 gm. per day), sucrose and milk was added. Insulin was given subcutaneously with each meal in sufficient amounts to keep the blood sugar below the renal threshold level. When the animals were in good nutritional condition, food and insulin were withdrawn. The fasting period was considered as starting at the time of the first omitted meal. At the end of the second day of fasting, the bladders were emptied by catheter and urine was collected under toluene for three consecutive twelve-hour periods. Catheterization was performed at the end of each period. One cubic centimeter of 1:1000 epinephrine was injected subcutaneously every three hours of the last period.

Urinary glucose was determined by the Shafter-Hartman method, nitrogen by the macro-Kjeldahl. Calculations were made as follows using values determined by Chaikoff and Weber. Liver glycogen: The liver was taken as 5 per cent of the body weight and was considered as containing 0.35 per cent glycogen (the maximum amount of glycogen found by Chaikoff and Weber in the liver of a fasted depancreatized dog after three

days of fasting). Protein: Each gram of nitrogen excreted during the epinephrine period in excess of that excreted during the previous twelve hours was considered a possible source of 6.4 grams of the "extra" glucose. Glycerol from fat: Chaikoff and Weber found that the caloric expenditure of the dogs during the twelve hour epinephrine period was 50 calories/kilo. If the dog was utilizing only fat for energy, the glycerol from this fat would

TABLE 1

DOG NUMBER	WEIGHT	LAST 12 HOUR PERIOD BEFORE EPINEPHRINE			1 CC. 1:1000 EPINEPHRINE SUBCUTANEOUSLY AT 3 HOUR INTERVALS				MUSCLE GLYCO- GEN	TOTAL AVAILABLE SOURCE OF EXTRA GLUCOSE AS GLUCOSE
		Glucose	Nitro- gen	D.N	Glucose	Nitro- gen	D:N	Extra glucose		
	<i>kilo</i>	<i>grams</i>	<i>grams</i>		<i>grams</i>	<i>grams</i>		<i>grams</i>	<i>grams</i>	
1	8.8	6.8	2.62	2.60	25.9	4.03	6.42	19.1	16.5	31.7
4	8.3	1.22	1.07	1.14	10.20	1.38	7.38	8.98	15.6	23.43
5	3	0.07	0.48	0.15	5.19	2.66	1.95	5.12	5.6	21.6
6	5.7	4.06	2.27	1.79	14.76	3.61	4.09	10.7	10.7	20.7
7	5	3.80	1.56	2.4	9.9	1.28	7.7	6.1	10.2	13.9
8	9.1	3.25	1.02	3.08	28.08	3.39	8.28	24.83	17.1	38.8
9	7	0.98	1.09	0.9	26.22	3.80	7.0	25.24	13.7	36.2
10	7	10.88	2.76	3.93	29.66	3.25	9.13	18.78	18.8	28.9
11	10	5.66	1.91	2.96	16.17	3.26	4.96	10.51	13.2	26.7
12	8	4.88	1.61	3.04	11.87	2.15	5.53	6.99	15.0	24.2
13	Samples lost				12.01	2.12	5.65		17.1	23.52
14	9.1	9.33	2.99	3.55	31.92	4.18	7.64	22.59	17.1	38.8
15	10	6.12	1.72	3.55	20.30	2.45	8.28	14.18	18.8	28.9
16	6.8	3.72	1.39	2.66	17.43	1.88	9.29	13.71	12.8	20.6
Average.....		4.80	1.71	2.44	18.63	2.84	6.63	13.16	13.9	25.8

TABLE 2

OBSERVERS	NUMBER OF DOGS	PIRE-EP- NEPHRINE D:N	NITROGEN EXCRETED DURING EPI- NEPHRINE PERIOD	GLUCOSE EXCRETED, EPI- NEPHRINE PERIOD	"EXTRA GLUCOSE", EPI- NEPHRINE PERIOD
			<i>grams/kgm.</i>	<i>grams/kgm.</i>	<i>grams/kgm.</i>
Chaikoff and Weber.....	7	6.5	0.41	4.9	3.1
Bollmann, Mann and Wilhelmj.....	4	3.2	0.39	2.2	1.2
This report.....	14	2.4	0.41	2.4	1.8

be a possible source of 0.53 gram per kilo of body weight. Muscle glycogen: If the muscles constitute 40 per cent of the body weight and contain at this time 0.47 per cent glycogen (determined by Chaikoff and Weber), muscle glycogen is capable of contributing 1.88 grams glucose/kilo body weight. "Extra" glucose was determined by subtracting the glucose excreted during the period just prior to the epinephrine period from the

glucose excreted during the epinephrine period. For example, dog 6 weighed 5.7 kilo, excreted 14.76 grams glucose with epinephrine stimulation and 4.06 grams in the period just previous or extra glucose amounting to 10.7 grams. Calculating the possible glucose from the aforementioned sources we find that liver glycogen may contribute $0.05 \times .0035 \times 5,700$ or 0.99 gram, glycerol may contribute 5.7×0.53 or 3 grams, protein (3.61 - 2.27) 6.4 or 1.34×6.4 or 8.58 grams, and muscle glycogen 1.88×5.7 or 10.7 grams.

RESULTS. As can be seen from table 1, in no case did the amount of "extra" glucose exceed the total possible source of glucose from sources other than fatty acids. In most cases the extra glucose could be accounted for either by glycogen alone, or by protein, glycerol and liver glycogen combined.

DISCUSSION. These results do not check with those obtained by Chaikoff and Weber. One point that may contribute to this discrepancy is the great difference in D:N ratio for the period immediately preceding the epinephrine stimulation (table 2). The average D:N ratio for this period as reported by Chaikoff and Weber was 6.5 while our average D:N ratio for the same time was 2.4. The amount of nitrogen in both cases is similar and the difference all lies in the glucose excretion which would indicate that Chaikoff and Weber's animals had not reached a fasting level and that glycogen stores were contributing a good share of the "extra" glucose.

Our "extra" glucose is almost equal to the average possible muscle glycogen, confirming Bollman, Mann and Wilhelmj whose results showed that muscle glycogen may possibly be the source of this "extra" glucose. It is interesting to note how closely the nitrogen values check in the three studies.

CONCLUSION

These experiments show that it is not necessary to postulate gluconeogenesis from fatty acids to account for the extra glucose excreted under the influence of epinephrine, and second, the average amount of extra glucose excreted was not more than could be derived from muscle glycogen as computed.

REFERENCES

- (1) CHAIKOFF AND WEBER. J. Biol. Chem. 76: 813, 1928.
- (2) BOLLMANN, MANN AND WILHELMJ. J. Biol. Chem. 93: 83, 1931.
- (3) CHAMBERS, HIMWICH AND KENNARD. J. Biol. Chem. 108: 217, 1935.

GLOMERULAR FILTRATION AND UREA EXCRETION IN RELATION TO URINE FLOW IN THE DOG

JAMES A. SHANNON

From the Department of Physiology, New York University College of Medicine

Received for publication May 29, 1936

Of chief historical interest in the study of the excretion of urea by mammals are the investigations of Austin, Stillman and Van Slyke (1921) and of Moeller, McIntosh and Van Slyke (1928) on man, which introduced the empirical concept of standard and maximum urea clearances. This concept was accepted for dogs by Jolliffe and Smith (1932a, b) and by Summerville, Hanzel and Goldblatt (1932), and it has been assumed in the recent studies of Van Slyke, Rhoades, Hiller and Alving (1934). Dominguez (1935) collected from the literature data on urea clearances in the dog and, adding some further observations furnished by Goldblatt, formulated an equation to describe changes in the urea clearance relative to the rate of urine formation. This equation was applicable at all urine flows, as opposed to the discontinuity of function implicit in the concept of standard and maximum clearances. Dominguez' treatment was also empirical, the fusion of standard and maximal clearances effected by him being accomplished by the arbitrary assumption that the maximum clearance of Van Slyke could be taken as the asymptote towards which the urea clearance approached as the urine flow increased, and which was approximated at the augmentation limit. This type of analysis is of uncertain value, since the indiscriminate admixture of data without regard for the conditions under which they have been obtained is apt to obscure important physiological variables. In 1925 Bourquin and Laughton presented data on dogs that could not be explained in terms of standard and maximum clearances. They studied cycles of diuresis and showed that, at any urine flow, when the urine flow was rising rapidly the urea clearance was usually higher than when the urine flow was falling. Their experiments were complicated, however, by the unacknowledged error of dead space, and also by a possibly inadequate method of emptying the bladder.

The present observations on the excretion of urea in the dog were undertaken with the hope that it might be possible to define more exactly the several factors operating in this important process. The central feature in this problem is the measurement of the rate of glomerular filtration.

Recent investigations in this laboratory, starting with the work of Jolliffe, Shannon and Smith (1932), have furnished the experimental basis for the use of the non-metabolized carbohydrate, inulin, for this purpose in the dog-fish (Shannon, 1934), the dog (Shannon, 1935) and man (Shannon and Smith, 1935). Supplementary evidence supporting this procedure has been furnished by Professor Richards and his co-workers, who were led independently by their experiments on the frog kidney to the study of the excretion of inulin in the dog (Richards, Westfall and Bott, 1934, 1936, Westfall and Hendrix, 1936).

The parenteral administration of inulin involves, however, the injection of relatively large quantities of saline, and its use is therefore sometimes disadvantageous in experiments in which one wishes to avoid any derangement of salt and water balance in the body. For this reason the creatinine clearance, which we have shown to be identical with the inulin clearance in the normal dog (Shannon, 1935, 1936) has been used as a measure of the filtration rate. The identity of the simultaneous inulin and creatinine clearances at low urine flows, where the U/P ratio is as high as 570, and data presented in this communication (fig. 5) lead us to believe that no error due to back diffusion of creatinine is introduced in the present experiments. (The observations of Van Slyke, Hiller and Miller (1935) on the excretion of ferrocyanide in the dog have been interpreted as supporting the belief that the creatinine clearance is at the level of glomerular filtration, but the great variability of the published results makes them of uncertain value. The standard deviation of the 27 creatinine-ferrocyanide comparisons was 18 per cent of the mean value of 0.96, a variability too large to indicate with certainty that there is no significant difference between these clearances.)

EXPERIMENTAL. We have proceeded on the assumption that different dogs might behave in a different manner with respect to the excretion of urea, and have therefore examined every dog completely, i.e., in respect to all variables. And on the further assumption that the same urine flow on different occasions might not be physiologically equivalent, even in the same animal, our experiments, unless otherwise indicated, have been so designed that the urine flow has varied throughout the entire physiological range in each series of observations. Each experiment has been so conducted that the rate of urine flow should return at the conclusion of the experiment, or at least once during the experiment, to the initial rate, or as near to the initial rate, as possible. (See table 1.) We believe that failure to observe these conditions is in part responsible for a misrepresentation of the true relation between the rate of water excretion and the urea clearance.

The urea and creatinine clearances have been examined in over 800

periods in 6 dogs. These animals were well trained females weighing from 17 to 28 kgm. Three of the dogs were observed upon a cracker meal, sucrose and lard maintenance diet, two upon a mixed diet, and one dog on both of these diets at different times. Adequate vitamins were given to all animals, and a salt mixture was used to supplement the low protein diet.

The general experimental procedures and chemical methods were the same as those that have been reported previously from this laboratory,

TABLE 1

A typical experiment (no. 37B, dog E, low protein diet) to illustrate the routine of the experiments performed on stationary and falling urine flows

Water was withheld from the animal for the 48 hours preceding the experiment. One hundred, 50, 50 and 50 mgm. per kgm. of creatinine were injected subcutaneously at the zero hour, 2:55, 5:14 and 7:44. Fifty and 15 cc. of water per kgm. were administered by stomach tube at 2:05 and 7:14.

PERIOD	TIME FROM ZERO HOUR	DURATION OF PERIOD	URINE FLOW	PLASMA CONCENTRATION		U/P RATIO		CLEARANCE		UREA CREATININE
				Urea	Creatinine	Urea	Creatinine	Urea	Creatinine	CLEARANCE RATIO
		minutes	cc./minute	mgm. per cent	mgm. per cent			cc./minute	cc./minute	
1	1:00-1:30	30	0.136	25.6	9.62	171.0	426.0	23.2	58.0	0.400
2	—2:05	35	0.143	25.6	9.84	164.0	416.0	23.4	59.5	0.393
3	3:10-3:40	30	4.10	23.7	11.4	8.64	15.4	35.5	63.1	0.563
4	—4:11	31	4.30	22.5	11.8	8.28	14.5	35.6	62.4	0.570
5	—4:40	29	4.10	21.3	11.9	8.44	15.1	34.6	61.9	0.559
6	—5:11	31	3.80	20.1	10.9	8.63	15.8	32.8	60.0	0.547
7	—5:43	32	3.03	19.0	9.4	10.6	19.6	32.1	59.3	0.541
8	—6:14	31	2.87	18.1		11.3		32.4		
9	—6:44	30	1.40	17.6	12.1	21.2	41.9	29.7	58.7	0.506
10	—7:17	33	0.88	17.2	12.1	31.6	66.1	27.8	58.2	0.478
11	—7:44	27	0.48	16.9	11.9	54.4	122.0	26.1	58.4	0.446
12	—8:14	30	0.250	16.8	10.3	98.8	237.0	24.7	59.2	0.417
13	9:20-9:50	30	1.63	15.7	11.4	19.6	37.9	32.0	61.8	0.518
14	—10:20	30	1.03	15.4	11.3	29.3	58.5	30.2	60.3	0.501

except in the following respects. In all instances where the rate of urine flow was below 2 cc. per minute, the bladder was washed out, as described by Shannon (1936). In some cases the creatinine determinations were done on iron filtrates (Steiner, Urban and West, 1932) of plasma and urine, and in others on diluted urines and tungstic acid filtrates of plasma as detailed by Shannon, Jolliffe and Smith (1932). In order to maintain the concentration in the plasma as constant as possible creatinine was administered in 8 to 10 per cent solution injected subcutaneously in circum-

scribed areas in doses of 50 to 100 mgm. per kgm. every 2.5 to 3.5 hours.¹ In the calculation of creatinine clearances no correction has been applied for endogenous creatinine; this correction would in any case be slight with plasma levels of 10 mgm. per cent, and constant for all periods.

EXPERIMENTAL RESULTS. For convenience of description the experimental results will be discussed as of two types. In the first type, observations were made while the urine flow was either constant or falling, the observation periods being removed at least one hour from the administration of water. The second type of experiment consists of observations in which the effect of rising urine flow was examined against a background afforded by the first type of experiment.

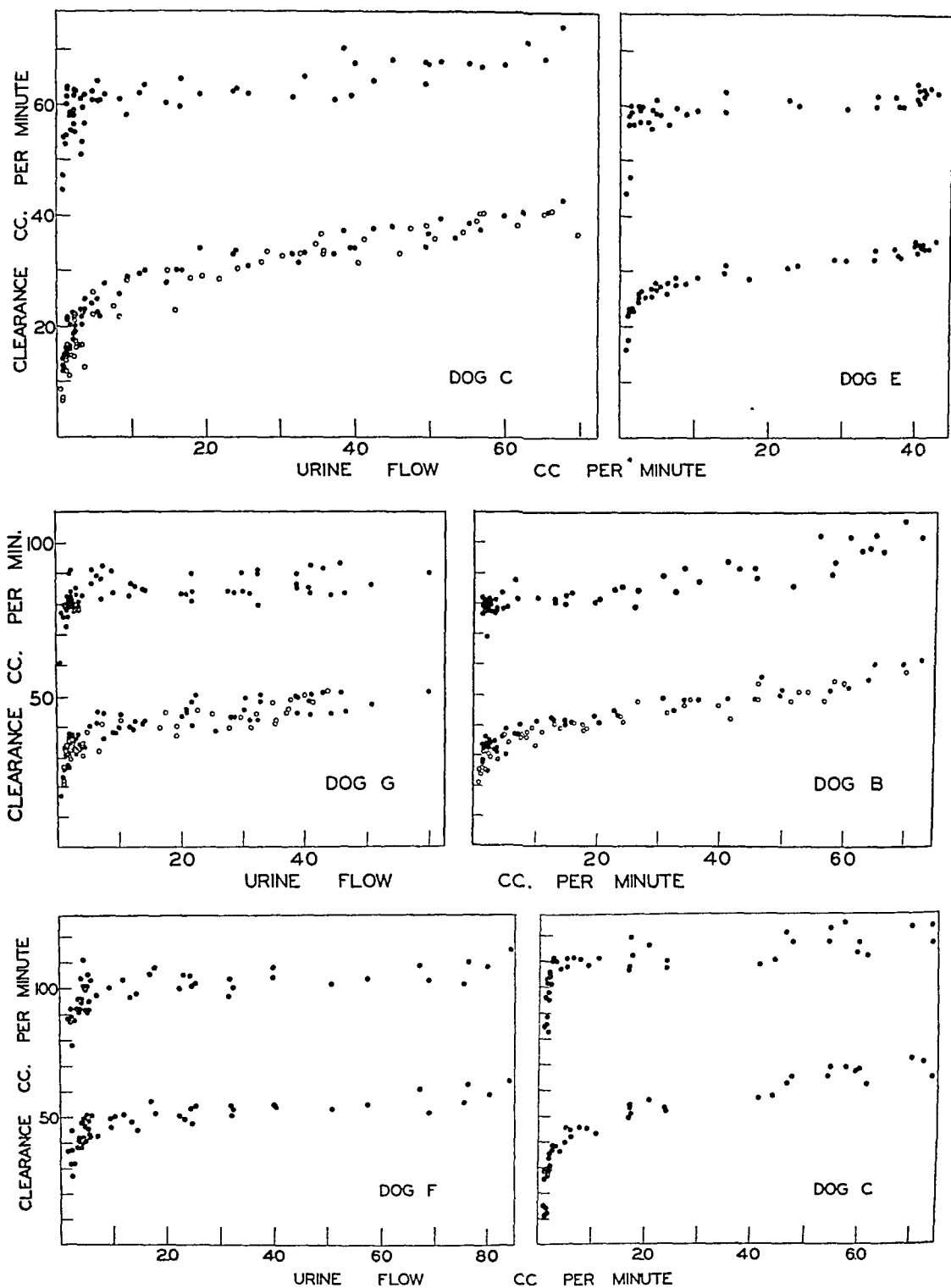
Observations on falling urine flows: Creatinine clearance. A typical experiment belonging to the first type is reported in full in table 1. Graphical summaries of all experiments of this first type (except those on dog A²) are given in figures 1 to 3, where the urea and creatinine clearances are plotted against the rate of urine formation.

The first significant fact demonstrated by these experiments is that the rate of glomerular filtration is essentially unrelated to the rate of water excretion within the range of ordinary experimental urine flows. This fact is revealed to better advantage by the summary of all the creatinine data given in figure 4. Each datum in the figure represents the mean of five clearance periods, selected by urine flow. For ease of comparison, the data on each dog have been adjusted to a percentage basis, taking the mean creatinine clearance at 0.5 to 1.0 cc. per minute as 100 per cent. It will be observed in figure 4 that between 0.5 and 4.0 cc. per minute the average creatinine clearance is essentially constant.³ At rates of urine formation above 4.0 cc. per minute there is a variable tendency for the filtration rate to increase. The maximal increase (relative to the rate at 0.5 cc. per minute) in the various dogs shown in this figure is 25 per cent for dog B, 13 per cent for dog C on low protein diet (dots) and 6 per cent on high protein diet (circles), 6 per cent for dog E, 8 per cent for dog F, 3 per cent for dog

¹ Any period following a large subcutaneous injection may show an aberrant urea clearance. For this reason we have administered creatinine dissolved in minimal quantities of water.

² No creatinine clearances were done on dog A, but the urea clearances would fall within the scatter of those of dog C on a low protein diet.

³ There is one significant fact that is lost when the creatinine clearances are averaged in the manner shown in figure 4. The first one or two periods at the peak of diuresis may be slightly higher than the mean value of the clearance when the urine flow is between 0.5 to 4.0; cc. per minute. This phenomenon is inconstant, and may appear regardless of where the peak of diuresis may be (i.e., 1 cc. or 8 cc. per minute). This may be similar to the phenomenon described by Jolliffe, Shannon and Smith (1932), which was attributed in part to the act of administration of water, as well as to excessive hydration when large volumes of water are given to obtain high urine flows.



Figs. 1-3

G. These changes in filtration rate are relatively small in contrast to an 800–1600 per cent increase in the rate of water excretion. Conversely, when the urine is below 0.5 cc. per minute the filtration rate tends to fall.⁴ These changes in glomerular filtration, because of their variability and the circumstances required to evoke them, may best be interpreted as due to incidental changes in renal circulation, or to excessive hydration or dehydration of the blood, rather than to a specific reaction of the glomerular apparatus. A possible exception to the above conclusion is evident in dog B, as shown in figure 2, which consistently showed a significant increase in the creatinine clearance with increasing urine flow on every occasion that it was examined. But even here the change in creatinine clearance is of a much smaller order of magnitude than the change in the rate of water excretion, and cannot be held to bear a causal relation to the latter.

Concerning the conclusion that the rate of water excretion is essentially independent of the rate of glomerular filtration, it may be noted that Peters (1935) has criticized this view and asserted that it was not warranted by any available evidence. We believe, however, that our present data are adequate to justify the assertion that in water diuresis in the dog there is no functional relationship between the rate of glomerular filtration and the rate of urine flow. This conclusion is based chiefly upon two facts. The first is the point made above, that in some animals wide variations in urine flow are not accompanied by significant changes in glomerular filtration. Second, in spite of the fact that under certain conditions changes in glomerular filtration may be accompanied by proportional changes in urine flow (thus keeping the U/P ratio of creatinine constant with small

⁴ We believe that the fall in the creatinine clearance at low urine flows is not due to back diffusion of creatinine, since we have demonstrated that the creatinine and inulin clearances remain identical at these same U/P ratios (Shannon, 1936). Moreover, when the creatinine clearance is depressed, because of excessive dehydration, and this dehydration is relieved by the administration of moderate amounts of water, the creatinine clearance returns to the general level with no essential change in the creatinine U/P ratio (see fig. 4).

Figs. 1–3. Creatinine and urea clearances in relation to urine flow, as observed when the latter is stationary or falling.

The data shown in these figures are the experimental observations that are further analyzed in figures 4 and 6. Each point is a single clearance period uncorrected for surface area. In each figure the upper data represent creatinine clearances, and the lower data represent urea clearances; the circles represent urea clearances in the absence of administered creatinine, the dots, urea clearances in the presence of administered creatinine. The data on dog C (fig. 1) and dogs E and B were obtained on a low protein diet, while those on dog C (fig. 3) and dogs F and G were obtained on a mixed diet.

The presence of exogenous creatinine in no way disturbs the relation of the urea clearance to urine flow, as observed without exogenous creatinine. (See dog C, fig. 1, dogs G and B, fig. 2.)

absolute changes in urine flow resulting), it is also true that wide changes in the former are sometimes not accompanied by any change in urine flow whatever (see fig. 5).

Urea clearance. In contrast to the constancy of the creatinine clearance, we find that the urea clearance increases systematically with the rate of urine formation throughout the entire range of urine flow, and no point may properly be designated as an augmentation limit, either in the sense of

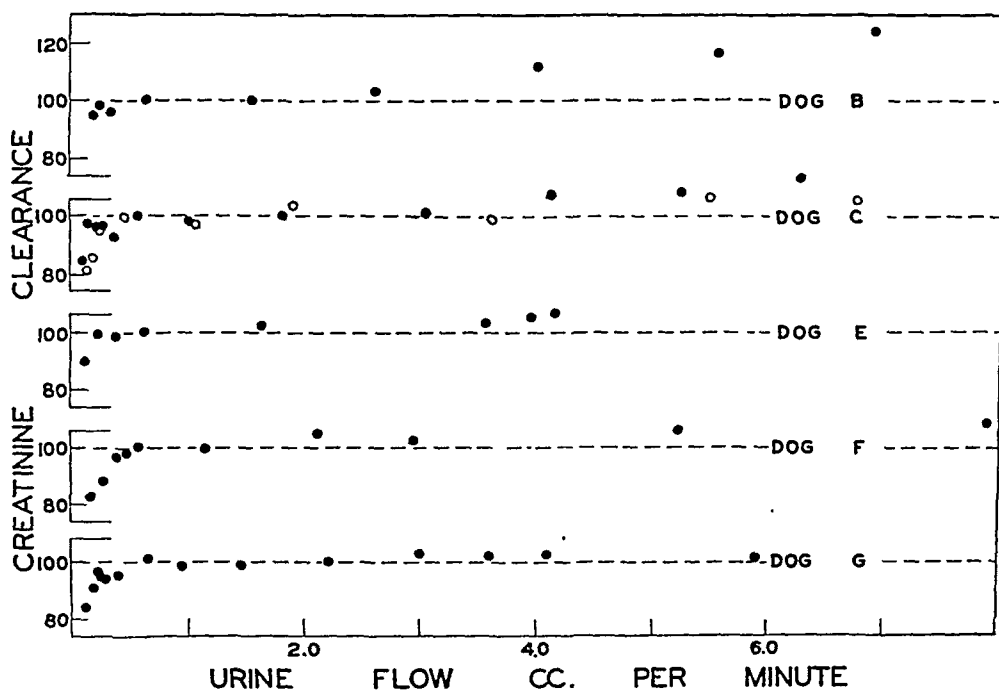


Fig. 4. Creatinine clearances (glomerular filtration) in relation to rate of water excretion.

The creatinine clearances of each dog, shown in figures 1 to 3, are averaged in groups of 5 according to urine flow and expressed on a per cent basis, taking the average values observed in each dog at urine flows of 0.5 to 1.0 cc. per minute as 100.

The creatinine clearance, which in the dog may be taken as equal to the rate of glomerular filtration, is essentially unrelated to urine flow between 0.5 and 4.0 cc. per minute. The decrease observed at urine flows below 0.5 cc. and the increase observed at urine flows above 4.0 cc. are probably referable to changes in circulation, blood hydration, etc., rather than to a specific reaction of the glomerular apparatus.

Austin, Stillman and Van Slyke (1921) or of Dominguez (1935). Examination of different dogs reveals that the rate at which the urea clearance changes with changing urine flow is quantitatively not the same in all animals. The rate of change is greatest in dog C (on both a low and high protein diet) and least in dog F (high protein diet).

The administration of creatinine does not alter the relationship between urea clearance and urine flow, as is demonstrated by the data on three

dogs, C, B, and G in figures 1 and 2. In view of this fact, we believe that observations on the urea and creatinine clearances made in experiments

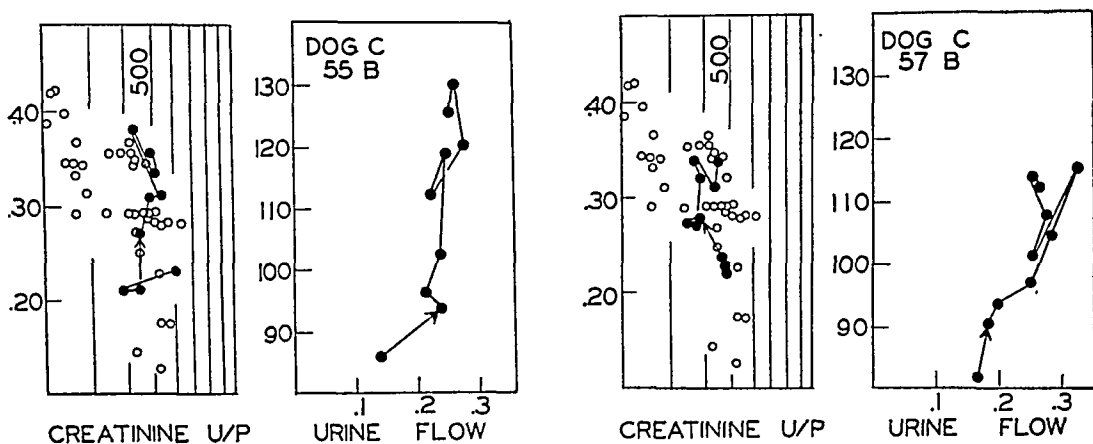


Fig. 5. Experiments showing the effect of slow hydration of a dehydrated dog on the creatinine and urea clearances, and on the urea/creatinine clearance ratios.

The relation between urea/creatinine clearance ratio and the log of the U/P ratio of creatinine is shown at the left in each instance. The open circles are the data shown for dog C in figure 4, as observed on a stationary or falling urine flow. The solid dots represent observations on a rising urine flow. The figures to the right relate creatinine clearance to urine flow in the same manner as in figures 1 to 3. The urine collection periods in these experiments were approximately 40 minutes in duration.

Experiment 55 followed 24 hours' abstinence from water. After each of the first 5 periods 50 cc. of water were injected subcutaneously.

Experiment 57 followed 48 hours' abstinence from water. After two control periods, 100 cc. of water were injected subcutaneously, and after each of the next 3 periods, 50 cc. of water were injected in the same manner.

During dehydration, the urea/creatinine clearance ratio appears to be depressed, relative to the "normal" values observed on a falling urine flow. Not only is the urea/creatinine clearance ratio depressed during dehydration, but the creatinine clearance itself is depressed below the "normal" values, as observed on a falling urine flow (by hemoconcentration, etc.). When hydration is effected slowly by the subcutaneous injection of water, both the creatinine clearance and the urea/creatinine clearance ratio rise; there is no great change in the U/P ratio of creatinine, and consequently little increase in urine flow. The fact that the creatinine clearance can increase over 30 per cent without a significant change in urine flow strengthens the belief that the rate of water excretion is within wide limits independent of the rate of glomerular filtration.

The rise in the urea/creatinine clearance ratio shows that the urea clearance increases to a much greater extent during slow hydration than does the creatinine clearance. This phenomenon is not the same as the exaltation of the urea clearance shown in figures 7 and 8.

where creatinine has been administered are applicable to the normal animal uncomplicated by creatinine injection.

Assuming that urea is freely filterable at the glomerulus (an assumption

for which there is available a large amount of evidence which need not be reviewed here) then the urea clearance should be equal to the rate of filtration unless some of the filtered urea is destroyed or reabsorbed by the renal tubules. It is well known that the urea clearance is lower than the glomerular clearance, presumably for one or both of these reasons; since there is no evidence to the contrary we will assume in the following discussion that the deficit in the urea clearance is due only to tubular reabsorption. And since the creatinine clearance in the dog may be taken as equal to the rate of glomerular filtration, the degree to which urea has been reabsorbed is indicated by the fraction $1.0 - \text{urea/creatinine clearance ratio}$, while the U/P ratio of creatinine indicates the degree to which the glomerular filtrate has been concentrated by the reabsorption of water. The relationship between urea reabsorption and water excretion is therefore best portrayed, for our present purpose, by plotting the urea/creatinine clearance ratio against the U/P ratio of creatinine, using the logarithm of the latter for convenience, since this term varies from 10 to nearly 800. The data presented in figure 6 are analyses made in this manner of the data shown in figures 1 to 3. (Attention is again called to the fact that these observations were all made under conditions where the urine flow was constant or falling.)

Three points can be established from these data. First, the urea clearance varies with urine flow in a systematic manner throughout the entire range of the latter. Although the relationship between these two variables differs considerably from one dog to another, the general trend between urea clearance and urine flow is the same in all dogs.⁵ Second, the degree of urea reabsorption at a given urine flow in a given dog is quantitatively the same when the rate of glomerular filtration is low (on a low protein diet) and when the rate of glomerular filtration is high (on a high protein diet). This fact was demonstrated with particular care in dog C. Third, at the highest urine flow obtainable (about 8 cc. per min.) a large fraction of the filtered urea is still reabsorbed.

So far as the first of the above points is concerned, we suppose that the essential factors in urine flow are the duration of time during which the concentrated urine is in contact with the tubules distal to the point of water reabsorption, and the tendency for the contained urea to diffuse outward.

⁵ The above description does not deny the general parallelism that has previously been noted in this and other laboratories between the urea and creatinine clearance under ordinary experimental conditions. In most reported experiments the urine flow has been relatively large, under which conditions there would be little variation in the urea/creatinine clearance ratio. The progressive increase in urea reabsorption noted here would not in any case be demonstrable except in experiments in which the changing urine flow was followed in a systematic manner. But where the urine flow is varied over a wide range, the urea/creatinine clearance ratio, or any measurement implied by this ratio (such as urea extraction ratio or urea clearance) must undergo wide variation in relation to urine flow.

Both these factors can be related to the U/P ratio of creatinine, and we note that the extent of urea reabsorption is related to the logarithm of this term. This relationship, with the exceptions noted below, appears to obtain in all dogs whether the urine is constant or falling, and regardless of diet, etc. For convenience we will refer to it as the logarithmic relationship, without implying any rigid mathematical description.⁶

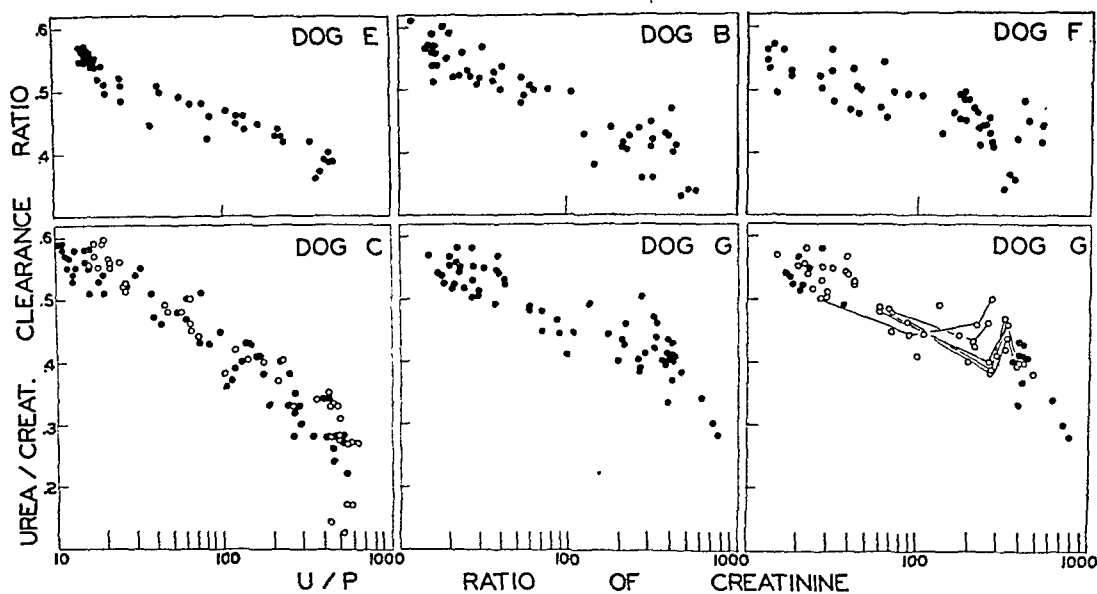


Fig. 6. The urea/creatinine clearance ratio in relation to the reabsorption of water, as indicated by the U/P ratio of creatinine.

Data as in figures 1 to 3.

The fraction of filtered urea that is reabsorbed is equal to $1.0 - \text{the urea/creatinine clearance ratio}$. The relationship shown here is apparently linear and indicates increasing urea reabsorption with increase in water reabsorption throughout the entire range studied. Diet in no way influences this relationship, as demonstrated specifically in the case of dog C.

At high urine flows the urea/creatinine clearance ratio is less than 0.6; if extrapolation is made to a U/P ratio of 1.0, this ratio would have a value no higher than 0.7. In the data of dog G, observations made at stationary urine flows are indicated by dots, and observations on a falling urine flow are indicated by circles. In 5 experiments, consecutive periods have been connected by lines.

Further inspection of figure 6 reveals that the scatter of the data is greater than is to be expected from experimental error, indicating that urea

⁶ This relationship is usually as evident in single experiments as in the mass plots shown in figure 6. An exception to this statement was observed in dog G. Here in some experiments the depression of the urea clearance on falling urine flows appeared to be related to the rate of deceleration of the urine flow, rather than the existing creatinine U/P ratio. But examination of the remainder of the data on this dog and of data obtained on the others did not support the suggestion that the rate of deceleration of the urine flow is itself a factor influencing urea reabsorption.

reabsorption may be affected by one or more factors other than those involved in urine flow. One such factor is dehydration. In dogs that have been deprived of water for 24 hours or more the urea clearance may be excessively depressed (i.e., below the "normal" value for a given urine flow). An instance of this nature is given in figure 5. This excessive depression can be corrected by administering water in such a manner (preferably subcutaneously) that the rate of urine formation is not increased (at least to no greater extent that can be accounted for by the accompanying increase in glomerular filtration, as shown in fig. 5). We call attention to this phenomenon at this time, without wishing to discuss it further, because it accounts in part for the broad scatter of the data at low urine flows.

In interpreting the logarithmic relationship shown in figure 6, it should be recognized that the U/P ratio of creatinine might be physiologically related to 1, the degree of hydration of the body cells, or of the renal tubules, as well as to 2, the concentration of hormones, proteins, etc., in the plasma. And this ratio is at least roughly indicative of 3, the extent of water reabsorption by the tubules; 4, the rate of fluid movement through the tubules, following water reabsorption and 5, the potential extent to which urea would have been concentrated in the tubules had there been no urea reabsorption.

It seemed to us that it should be possible to rule out of consideration some of these factors. For example, to induce diuresis (3, 4 and 5) independently of hydration and blood composition (1 and 2), we have used the intravenous administration of 50 per cent sucrose solution, with and without the simultaneous administration of pitressin. An experiment of this type is shown in figure 8, experiment 84B.⁷ In this and other such experiments the logarithmic relationship has been maintained, from which we conclude that it depends upon 3, 4, and 5, or upon some other local renal condition, rather than upon the systemic factors.

Observations on rising urine flows. It was early observed that the urea clearance tends to rise to abnormally high values, relative to the creatinine clearance, when the urine flow is increasing rapidly. A series of experiments examining this phenomenon in relation to water diuresis is given in figure 7. This figure includes experiments illustrating the extent to which the rate of acceleration of the urine flow, the original rate of urine flow, and the amount of water given, are related to this exaltation of the urea clearance.

⁷ This does not imply that systemic factors have no influence upon urea reabsorption, as has been indicated by the observations of others (see Poulsson, 1930 and the experiments reported here, particularly experiment 85B, fig. 8). The point under discussion concerns only whether or not these factors (in contradistinction to local renal factors) are responsible for the progressive increase in urea reabsorption that is related to decreasing urine flow.

If the urine flow is increased slowly, the logarithmic relationship previously described may be exactly retraced. This is demonstrated in experiment 26B, where the rate of urine formation was caused to increase from 0.14 cc. to 3.74 cc. per minute by the slow administration of water over a period of seven hours. But in 18 attempts this was the only instance in which so slow an acceleration of the urine flow could be obtained.

With more rapid acceleration of urine flow experiment 28B was obtained. Although, in this instance, the logarithmic relationship was approximately retraced, there is an evident tendency for the urea clearance to rise, relative to the creatinine clearance, during the early part of the acceleration of urine flow.⁸

The most frequent result obtained on an increasing urine flow is of the nature of that shown by experiment 70B and table 2; the urea clearance rises markedly, relative to the creatinine clearance, and out of all proportion to the change in urine flow, this exaltation being transient in the sense that the urea/creatinine clearance ratio may return to "normal" before the peak of diuresis is reached. The result obtained in experiment 72B followed the administration of 10 cc. of water per kgm. *per os*, which led to an immediate but small rise in urine flow, which then returned quickly to its previous level. The urea/creatinine clearance ratio increased abruptly, maintained the abnormally high level for two periods and then fell as the urine flow decreased. Fifty cubic centimeters of water per kilogram were then administered *per os*, and resulted in a second abrupt increase in the urea/creatinine clearance ratio, the increase being maintained as the urine flow was rising, but returning after the peak of diuresis to the normal level. Under certain conditions (exp. 74B) the exaltation of the urea clearance may persist as long as the urine flow continues to rise, but there

⁸ We are convinced that these variations in the urea/creatinine clearance ratio are not due to errors introduced by the dead space of the pelvis and ureters of the kidney. Our experiments have been so designed that the plasma level of creatinine has remained very constant throughout all periods of observation (see tables 1 and 2); and dead space, though it might affect the absolute values of both clearances, would in no way affect the urea/creatinine clearance ratio. The extent to which dead space would alter the absolute value of a clearance will be determined by the rate of acceleration of urine flow, the initial and final U/P ratios, and the duration of the period of observation. The exact evaluation of the dead space error would be possible from our creatinine data if this clearance were constant throughout the experiment, but unfortunately this is not the case. The creatinine clearance is usually depressed to some extent in the control periods (at low urine flows) and increases after hydration of the animal (figs. 4 and 5); there is also an inconstant increase in creatinine clearance following the administration of water, as mentioned above. But the fact that occasional, rapid increases in urine flow, starting at low values, may not be accompanied by significant changes in the creatinine clearance suggests that the dead space in the normal kidney of an upright dog is relatively small. The apparent maximum dead space, calculated from the experiment detailed in table 2, is 0.5 cc.

is no relationship between the maximal exaltation and the peak of diuresis.

Eighteen experiments of this general type were performed, and in only the one case mentioned (26B) did the urea/creatinine clearance ratio fail to rise excessively on a rising urine flow. In general the rise in this ratio is most marked when the initial urine flow is low; we have been unable to obtain it when the initial urine flow was 2 cc. or higher (see table 2). It can be repeatedly evoked in a continuous experiment, as is shown by experiment 71B.

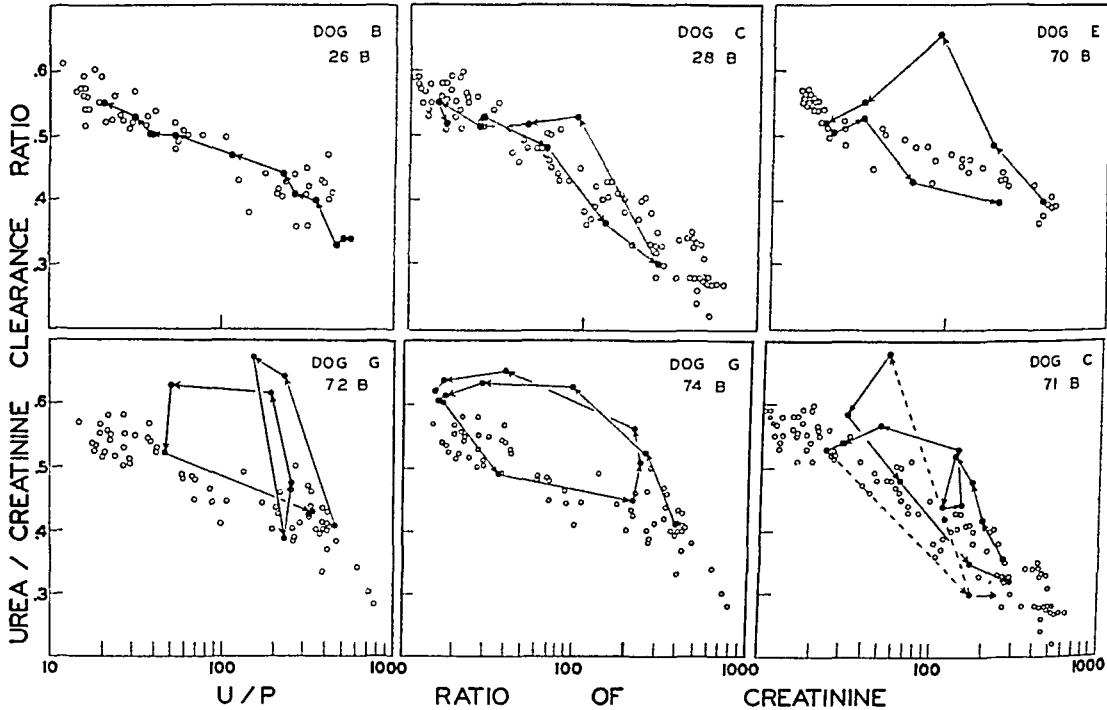


Fig. 7

It seemed possible that the phenomenon of the exaltation of the urea clearance on rising urine flow might be due to sudden hydration of cells of the renal tubules. This point was examined in six experiments, three of which are shown in figure 8. In these experiments control periods were taken at low urine flows; varying amounts (6 to 100 cc.) of 50 per cent sucrose were then injected intravenously, which caused an acute diuresis. The urea clearance rose relative to the creatinine clearance, just as though the urine flow had been increased by the oral administration of water. We must suppose that the renal tubules are at least relatively impermeable to sucrose, and that the presence of this substance in the interstitial fluid and the tubular urine would tend to dehydrate the tubule cells, in so far as it has any effect at all on local water equilibrium. In other experiments, in

which there was no exaltation of the urea clearance, the urine flow was maintained at a low level by the subcutaneous injection of 1 cc. of pitressin in a 20 kgm. dog, while rapid hydration of the body was effected by the

Fig. 7. The exaltation of the urea clearance during water diuresis.

The circles represent observations on stationary or falling urine flows, as recorded in figure 6. The dots represent observations during water diuresis. The arrows indicate the sequence of the experimental periods.

Experiment 26B, dog B (low protein diet). Water was withheld for 24 hours previous to the experiment. Following three control periods water was administered in quantities of 7 cc. per kilo *per os* every half-hour. The urine flow started to rise in 2.5 hours and during the next four hours maintained a steady increase. The logarithmic relationship between the urea/creatinine clearance ratio and U/P ratio of creatinine was exactly retraced. The periods were approximately 30 minutes in duration. The creatinine clearance was constant throughout.

Experiment 28B, dog C (low protein diet). Water *ad lib* previous to experiment. One hour before the beginning of period one, 15 cc. of water per kilo were administered by mouth. At the end of the fourth and sixth periods, there were given 7.5 and 25 cc. of water per kilo, respectively. The urea/creatinine clearance ratios observed on the rising urine flow approximated the "normal" values, except for the first period of diuresis, in which there was a slight exaltation. The periods in this experiment varied from 30 to 40 minutes. The creatinine clearance, after remaining fairly constant throughout the first seven periods (57.9-63.0), increased to 71.7 and 67 during the last two periods.

Experiment 70B, dog E (low protein diet). Water *ad lib* prior to the experiment. Following a control period, 12.5 cc. per kilo of water *per os* were given and observations were continued during the next three hours. The periods were approximately 20 minutes in duration. This is the most characteristic response obtained. The creatinine clearance rose steadily throughout the first five periods (50.8-58) and gradually returned to the control level.

Experiment 72B, dog G (high protein diet). Water *ad lib* prior to the experiment. This experiment is described in detail in the text. The periods were approximately 20 minutes in duration. The creatinine clearance rose during the fourth period (68.7-78.0), reached a peak of 86 in the eighth period and returned to 76 during the last two.

Experiment 74B, dog G (high protein diet). Water *ad lib* prior to experiment. Forty cubic centimeters of water per kilo were given after the first period and 20 cc. per kilo after the tenth period. The periods were approximately 20 minutes in duration. No significant change occurred in the creatinine clearance.

Experiment 71B, dog C (high protein diet). Water *ad lib* prior to the experiment. At the beginning of the first period 12 cc. of water per kilo were administered and the sixth period was followed by 25 cc. of water per kilo. The marked fall in the eleventh period in both urine flow and the urea/creatinine clearance ratio was spontaneous, as was the following rise (see dotted lines). The urine flows in these three periods were 3.75, 0.571 and 1.57. The arrow indicates that the true mean U/P ratio of creatinine in the eleventh period was much lower than was actually observed; while most of the water was excreted at a high urine flow, the urea in large part must have been excreted at a urine flow significantly below 0.571 for this figure to be the mean value for water excretion during this period. The periods were approximately 20 minutes in duration. The creatinine clearance rose irregularly throughout the first eleven periods (60-93) and then decreased to 83.

administration of 50 cc. of water per kgm. These conditions should effect the hydration of the renal tubules locally, as well as the body cells generally. That pitressin does not specifically prevent the exaltation of the urea clearance is shown in experiment 85B. In this experiment 1.0 cc. of pitressin was given subcutaneously and, after a control period, diuresis was induced by the intravenous administration of sucrose (5 grams in 10 cc. of solution). We infer from these experiments that neither pitressin nor the hydration of the body as a whole, or of the renal cells in particular, is the primary

TABLE 2

A typical experiment (no. 78B, dog E, low protein diet) to illustrate the exaltation of the urea clearance on rising urine flows caused by the administration of water

Note that when the urine flow was caused to increase from a high value no exaltation was found. This latter acceleration was slight when compared to that occurring from low urine flows. One hundred and 50 mgm. per kilogram of creatine were given at zero hour and 3:30. Fifty and 30 cc. of water per kilogram were administered by stomach tube at 1:13 and 4:35.

PERIOD	TIME FROM ZERO HOUR	DURATION OF PERIOD	URINE	PLASMA CONCENTRATION		U/P RATIO		CLEARANCE		UREA CREATININE
				Urea	Creatinine	Urea	Creatinine	Urea	Creatinine	CLEARANCE RATIO
		minutes	cc./minute	mgm. per cent	mgm. per cent			cc./minute	cc./minute	
1	40-1:13	33	0 109	8 10	7 58	172 0	479 0	18 8	52 2	0 360
2	—1:39	26	0 154	8 65	8 43	154 0	326 0	23 7	50 2	0 472
3	—2:01	22	1 14	8 98	8 43	33 9	51 1	38 7	58 3	0 664
4	—2:27	26	3 23	9 27	8 50	9 09	17 6	29 4	56 9	0 516
5	—2:54	27	4 07	9 43	8 58	7 93	14 7	32 3	59 8	0 540
6	—3:24	30	3 10	9 38	7 79	9 49	18 9	29 4	58 6	0 502
7	—3:49	25	2 1	9 24		11 85		24 9		
8	—4:09	20	2 6	9 12	9 63	10 06	21 9	27 6	57 0	0 484
9	—4:35	26	2 31	8 95	10 3	11 3	24 5	26 1	56 6	0 461
10	—4:55	20	2 45	8 74	11 36	11 1	22 3	27 2	54 6	0 498
11	—5:15	20	3 05	8 60	11 58	9 82	18 3	29 9	55 8	0 536
12	—5:39	24	3 33	8 50	11 43	8 97	17 1	29 9	57.0	0 525
13	—6:06 5	27 5	3 27	8 40	10 82	9 57	17 5	31 8	57 2	0 556

factor concerned in this phenomenon. Rather it appears that the exaltation of the urea clearance is directly related to an abrupt change in the extent of water reabsorption, as reflected in the U/P ratio of creatinine, or to the rate of water excretion, considered mechanically.

DISCUSSION. It is unnecessary to amplify the discussion of the experiments reported above except in respect to urea. It would appear that the concept of an augmentation limit relative to the excretion of urea in dogs is erroneous. The apparent existence of an augmentation limit in previous

data has arisen from two circumstances; first, the relationship between the urea clearance and urine flow (at constant and falling flows) varies quantitatively from one animal to another, and the massing of data from different animals, even though the data were collected under suitable conditions,

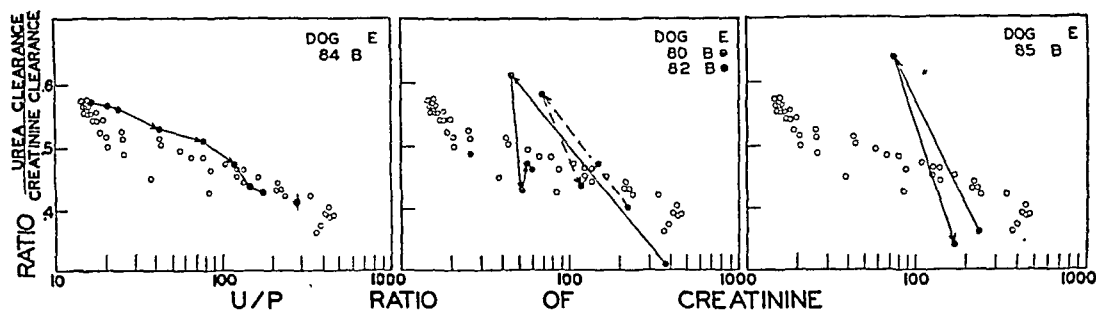


Fig. 8. Data showing the effect of sucrose solutions intravenously, with and without pitressin.

Experiment 84B, dog E (low protein diet). Water *ad lib* prior to experiment. Following one control period 25 grams of sucrose in 50 cc. of solution were administered intravenously. The urea and creatinine clearances were then determined during the following 4 hours. Periods were approximately 30 minutes in duration. The creatinine clearance fell following the injection of sucrose and then returned to its initial level in the third period, thereafter remaining constant (53.2–43.1–52.7 cc. per minute which was maintained).

Experiment 80B, dog E (low protein diet). Prior to the experiment water was withheld for 36 hours. Following one control period 10 grams of sucrose in 21 cc. of solution were given intravenously. Periods were approximately 20 minutes in duration. Creatinine clearances were 50.8, 41.1, 54.6, 57.8 and 58.3.

Experiment 82B, dog E (low protein diet). Water *ad lib* prior to the experiment. Following one control period 5 grams of sucrose in 10 cc. of solution were injected intravenously. Periods were approximately 20 minutes in duration. Creatinine clearances were 47.3, 49.7, 49.8 and 54.4.

Experiment 85B, dog E. Water *ad lib* prior to the experiment. One half hour before the beginning of the first period, 1 cc. of pitressin (10 pressor units, Parke, Davis & Co.) was given subcutaneously. Following the first period 3 grams of sucrose in 6 cc. of solution were administered intravenously. Periods were approximately 20 minutes in duration. Creatinine clearance was 43.4, 46.9, 46.4. Note the depression in urea/creatinine clearance ratio in the control period and following the exaltation.

It should be noted that the maximum urea/creatinine clearance ratio observed in these experiments is no indication of the true maximum actually reached; the period showing exaltation is usually preceded and followed by periods in which this ratio is relatively low, and this fact tends to obscure the real magnitude of the rise.

would tend to obscure the true relationship. Secondly, the phenomenon of the exaltation of the urea clearance on rising urine flows, which appears only under these specific conditions, tends to give maximum urea clearances at intermediate or low urine flows, and thus tends to obscure the true downward drift of the urea clearance, as described in this paper.

The exaltation of urea clearance on rising urine flows is undoubtedly the same phenomenon as was observed by Bourquin and Laughton (1925). Although we cannot explain its mechanism we believe that the phenomenon is a physiological one—i.e., having eliminated the mechanical error of tubular, pelvic and ureteral dead space, we must assign its genesis to the cells of the renal tubules. It can be evoked when the flow of urine is increased in the normal diuretic response to water, as well as when diuresis is evoked osmotically by intravenous sucrose, and it apparently does not depend on the state of hydration of the body. The facts that it is transient, lasting not longer than one hour, that the maximal urea clearance does not coincide with the maximal diuresis, and that it may be absent if the urine flow is increased slowly, indicate that it is the acceleration of the rate of urine flow that evokes the phenomenon rather than some change in the renal tubules, physiologically associated with decreased water reabsorption.

The most important aspect of our data concerns the question of why urea is reabsorbed in the mammalian kidney. (We set aside here as a feature secondary to the main problem, the excessive reduction of the urea clearance occurring at very low urine flows in dehydrated animals.) It was suggested by Rehberg (1926) and Holten and Rehberg (1931) that the progressive depression of the urea clearance as the urine flow was lowered was due to passive diffusion, in consequence of the increase in the concentration gradient across the tubules resulting from water reabsorption. But, in the light of our present results, it is impossible to accept this simple explanation. In a simple physical system, the change in concentration of any solute, due to diffusion across a permeable membrane, will be exponentially related to time. Given a constant creatinine clearance in the kidney, and assuming that the volume of the tubule distal to the site of water reabsorption remains constant, the U/P ratio of creatinine can be taken as a term directly proportional to the time during which a unit volume of urine remains in contact with a unit area of the tubular surface. Therefore, substituting $k \times U/P$ of creatinine for t in the ordinary diffusion equation, and letting $U-P$ represent the diffusion gradient, this equation becomes:

$$K = \frac{1}{U/P} \cdot \log \frac{1}{1 - \frac{a}{1 - \frac{P}{U}} \cdot \left(1 - \frac{Vu}{Vb}\right)}$$

Here U and P refer to the concentration of creatinine in bladder urine and plasma, a is 1—urea/creatinine clearance ratio, and Vu and Vb , respectively, the volumes of the urine and blood opposed to each other across the permeable surface.

In the equation as written above, it is supposed that the entire reab-

sorption of urea occurs distal to the site of reabsorption of water, and is dependent on the diffusion gradient of urea, essentially as suggested by Rehberg. But in addition the equation does, and must, take into account the time during which the concentrated urine is in contact with the tubule cells (a factor proportional, as we remarked above, to the creatinine U/P ratio), for at all urine flows this time element is a major factor determining the relative amount of urea reabsorbed.

But even in this amended form the diffusion hypothesis can not be made to fit the experimental data at both high and low urine flows. If the deficit in the urea clearance at a creatinine U/P ratio of 10 (which deficit amounts to 40 per cent) is due to a process of passive diffusion which is uniform with the process occurring at low urine flows, then at a creatinine U/P ratio of 100 the time of contact is by prediction long enough for diffusion to effect the approximately equal distribution of urea between blood and urine. A further increase in the creatinine U/P ratio should therefore not be accompanied by a further deficit in the urea clearance. The fact that there is no evident tendency for the urea/creatinine clearance ratio to level off before, or to become constant at creatinine U/P ratios of 100 would indicate that the simple diffusion hypothesis as stated above is inadequate. Apparently factors other than the diffusion gradient of urea and the time of contact are involved.

It is improbable that the failure of the simple diffusion hypothesis is due to our initial assumption of a constant tubular volume, *per se*, for any change in V_u of whatever magnitude would not obviate the predicted equilibrium between tubular urine and plasma at a creatinine U/P ratio of 100. But a change in V_u would affect both the extent of the permeable surface and the time of contact, and might affect K by altering the specific permeability of the tubule cells. It seems unlikely that the first two factors, which oppose one another, would be significant, though changes in the specific permeability of the tubule cells might well play an important rôle.

But as a second and perhaps more attractive hypothesis, it may be supposed that the reabsorption of urea involves two or more processes, or mechanisms, which are physiologically independent of each other. The deficit in the urea clearance observed at high urine flows may in part be of different genesis than the progressively increasing deficit occasioned by raising the creatinine U/P ratio above 10 (i.e., lowering the urine flow below 6 to 8 cc. per minute), which latter phenomenon has the appearance of increasing diffusion due to prolongation of the time of contact. But at the present moment no choice between these alternatives can be made on the available evidence. We feel that it is better to forego speculation in the hope that a new experimental approach will resolve some of the questions involved.

It is obviously impossible to interpret the excessive depression of the

urea clearance in dehydrated animals, or the exaltation on rising urine flows, until more is known about the fundamental factors involved in the major reabsorptive process or processes.

I wish to express my appreciation of the interest and critical assistance of Professor Homer W. Smith throughout this work.

SUMMARY

1. The urea clearance in relation to urine flow has been examined under a variety of conditions in six dogs (824 clearance periods), the simultaneous creatinine clearance being used in the majority of the periods in five dogs to measure the rate of glomerular filtration.

2. The rate of glomerular filtration is essentially constant and unrelated to the rate of urine flow in the ordinary experimental range of the latter. It may, however, be depressed by dehydration, or elevated by the administration of large doses of water.

3. At the highest urine flow obtainable (creatinine U/P ratio = 10) about 40 per cent of the urea filtered is reabsorbed. As the urine flow decreases an increased fraction of the filtered urea is reabsorbed; the increase in the reabsorbed fraction being approximately related to the logarithm of the creatinine U/P ratio.

4. Diuresis following a low urine flow is accompanied by a marked, transient exaltation of the urea clearance relative to the creatinine clearance, which may disappear before the peak of diuresis is reached. This exaltation is evoked by osmotic diuresis in a normal or pituitrinized dog, as well as by water diuresis in the normal.

5. The concept of an augmentation limit with its corollary of standard and maximum clearances does not appear to be applicable to the excretion of urea in the dog.

6. It is pointed out that a simple diffusion hypothesis, positing that urea escapes from the tubule distal to the point of reabsorption of water, and in consequence of the concentration gradient created by this reabsorption, is inadequate to explain the observed relationships between the deficit in the urea clearance and the rate of urine formation.

REFERENCES

- AUSTIN, J. H., E. STILLMAN AND D. D. VAN SLYKE. *J. Biol. Chem.* **46**: 91, 1921.
BOURQUIN, H. AND N. B. LAUGHTON. *This Journal* **74**: 436, 1925.
DOMINGUEZ, R. *This Journal* **112**: 529, 1935.
HOLTEN, C. AND P. B. REHBERG. *Acta Med. Scand.* **74**: 479, 1931.
JOLLIFFE, N., J. A. SHANNON AND H. W. SMITH. *This Journal* **101**: 639, 1932.
JOLLIFFE, N. AND H. W. SMITH. *This Journal* **98**: 572, 1931a.
This Journal **99**: 101, 1931b.
MØLLER, E., J. F. MCINTOSH AND D. D. VAN SLYKE. *J. Clin. Invest.* **6**: 427, 1928.
PETERS, J. P. *Body water*. Charles C. Thomas, 1935.

- POULSSON, L. T. *Ztschr. ges. exper. Med.* **71**: 576, 1930.
- REHBERG, P. B. *Biochem. J.* **20**: 447, 1926.
- RICHARDS, A. N., B. B. WESTFALL AND P. A. BOTT. *Proc. Soc. Exper. Biol. and Med.* **32**: 73, 1934.
Proc. Am. Physiol. Soc., This Journal **116**: 128, 1936.
- SHANNON, J. A. *J. Cell. and Comp. Physiol.* **5**: 301, 1934.
This Journal **112**: 405, 1935.
This Journal **114**: 362, 1936.
- SHANNON, J. A., N. JOLLIFFE AND H. W. SMITH. *This Journal* **102**: 534, 1932.
- SHANNON, J. A. AND H. W. SMITH. *J. Clin. Invest.* **14**: 393, 1935.
- STEINER, A., F. URBAN AND E. S. WEST. *J. Biol. Chem.* **98**: 289, 1932.
- SUMMERVILLE, W. W., R. F. HANZAL AND H. GOLDBLATT. *This Journal* **102**: 1, 1932.
- VAN SLYKE, D. D., A. A. HILLER AND B. MILLER. *This Journal* **113**: 611, 1935.
- VAN SLYKE, D. D., C. P. RHOADS, A. HILLER AND A. S. ALVING. *This Journal* **109**: 336, 1934.
- WESTFALL, B. B. AND J. P. HENDRIX. *Proc. Am. Physiol. Soc., This Journal* **116**: 160, 1936.

THE EFFECT OF ACUTE HEMORRHAGE ON THE EMPTYING TIME OF THE STOMACH¹

EDWARD J. VAN LIERE, CLARK K. SLEETH AND DAVID NORTHUP

*From the Department of Physiology, West Virginia University, Morgantown,
West Virginia*

Received for publication June 1, 1936

In 1933 Van Liere, Crisler and Robinson reported that anoxemia produced a prolongation of gastric emptying time in the dog. In 1936 the senior author with the aid of Lough and Sleeth using man as a subject obtained comparable results with those found in the dog. It seemed logical to assume that if anoxemia produced a delay in gastric emptying time, anemia would probably do likewise, as the latter condition produces anoxia.

In order to test the validity of this hypothesis, however, it was deemed worth while to study the effect of hemorrhage on the gastric emptying time. This study seemed important as man often suffers more or less severe losses of blood from various causes.

METHODS. The experiments were performed on normal human beings and dogs.

A. Man. The normal gastric emptying time was ascertained fluoroscopically on four healthy male medical students. The standard meal was given at 8:30 a.m. These subjects had had no food since the evening before. The meal was prepared as follows: 15 grams of Quaker Farina were added to 350 cc. of water; this mixture was cooked until the total volume was 200 cc. One gram of salt was added for flavor. After the meal had cooled, 50 grams of barium sulphate were added so that the gastric contents could be seen with the fluoroscope. A number of control observations were made on each individual. The average of these figures was used for the norm.

The effect of hemorrhage was now studied. One-tenth of the calculated blood volume was withdrawn from the subject. It was assumed for the purpose of these experiments that the blood constituted 7 per cent of the total body weight. Using this figure, a man who weighed approximately 150 pounds (68 K.) was bled 500 cc. After the blood had been withdrawn, the subject ate the standard meal and the gastric emptying time was again determined. Attention was paid to all details necessary for carefully controlled work.

¹ Aided by a grant from the Committee on Scientific Research of the American Medical Association.

B. *Dogs.* The normal emptying time of the stomach was determined in two vigorous dogs. The standard meal used in the dog was different from that used in man, as in the past 5 years a great deal of data on the normal gastric emptying time in the dog has been obtained using this particular meal (Van Liere, Crisler and Wiles). It was made up of the following ingredients: 40 grams of hamburger steak, 10 grams of dried ground bread and 50 cc. of milk; to this were added 15 grams of barium sulphate. A number of normal observations on the gastric emptying time were made and the average of these figures was used for the control. As was done in man, one-tenth of the calculated blood volume was withdrawn. The dogs were lightly anesthetized while the blood was being withdrawn. About an hour after they had been bled they were allowed to eat the standard meal and the gastric emptying time was again determined.

After the animals had entirely recovered from the hemorrhage (several weeks later) the effect of ether anesthesia on the gastric emptying time was determined. They were lightly anesthetized for the same length of time as they had been when the blood was withdrawn. About an hour after they had recovered from the anesthetic they were given the standard meal. It was found that the gastric emptying time was prolonged 38.5 per cent in the first animal and 29.6 per cent in the other. The next day, however, the gastric emptying time was normal in both animals.

RESULTS. The accompanying tables show the results obtained.

The data in tables 1 and 2 clearly show that hemorrhage has a distinct effect on the gastric emptying time. The average delay in the gastric emptying time in the 4 human subjects who ate the standard meal immediately after the blood had been withdrawn was 41 per cent. The meal eaten 24 hours later showed a delay in three subjects from 15 to 20 per cent. One man showed a normal emptying time at the end of 24 hours. For all practical purposes gastric motility in man returned to normal in all the subjects after 48 hours.

Dogs showed a greater prolongation of the gastric emptying time than did man. On this account it was necessary to delay giving the second standard meal for 24 hours. In the dog it was found that gastric motility returned to normal on the fourth day instead of the third day as in man.

DISCUSSION. In evaluating the data obtained in these experiments on man the psychic factor must be considered. It is admitted that it is impossible to rule this out. It can be said, however, that the subjects used in this work were young men who were familiar with the minor operative procedures employed and they looked upon the experiments as an ordinary routine procedure and an interesting experience. Two of the subjects who volunteered for this work had been used the previous year in studying the effects of low oxygen tensions on the gastric emptying time. There was far more danger in this latter experiment than in those reported in this

paper. The fact that gastric evacuation was still delayed on the second day showed that the psychic factor probably was not a very important one.

Hemorrhage caused the gastric emptying time of the dogs to be greatly prolonged. As mentioned earlier, the ether anesthesia was responsible for some delay for control experiments showed that ether anesthesia was capable of causing a delay in the gastric emptying time of about 35 per cent. This figure, however, is relatively unimportant as the gastric emptying time was delayed over 300 per cent after the blood had been withdrawn.

TABLE 1

The effect of hemorrhage on the gastric emptying time in man

NUMBER	NORMAL		IMMEDIATELY		24 HOURS LATER		48 HOURS LATER	
	Hours	Per cent	Hours	Per cent	Hours	Per cent	Hours	Per cent
1	2 0	100	3 0	150	2 0	100	2 0	100
2	1 7	100	2 5	147	2 0	117	1 5	88
3	2 6	100	3 3	127	3 0	115	2 7	104
4	2 5	100	3 5	140	3 0	120	2 7	108
Average	2 2	100	3 1	141	2 5	113	2 2	100

TABLE 2

The effect of hemorrhage on the gastric emptying time in the dog

NUMBER	NORMAL		IMMEDIATELY		48 HOURS LATER		72 HOURS LATER		96 HOURS LATER	
	Hours	Per cent	Hours	Per cent	Hours	Per cent	Hours	Per cent	Hours	Per cent
1	6 4	100	21 5	336	14 0	219	8 0	125	6 5	103
2	6 7	100	20 7	309	13 5	202	7 5	112	6 7	100
Average	6 55	100	21 1	322	13 7	210	7 7	118	6 6	101

Ether, furthermore, could not account for the tremendous delay in the emptying time observed after 48 hours.

The standard meal given the dog contained a good deal of fat and protein; this meal could not be compared to the carbohydrate meal which was given the human subjects. Another factor might be mentioned, that is, gastric secretion is probably diminished after a hemorrhage and this may have delayed the stomach emptying time. At the present time this phase is being studied.

The results reported in this paper correlate very well with the results obtained when working with low oxygen tensions, which work has already been mentioned. It is felt that the same mechanism is involved, namely,

the loss of blood caused an anoxia. The mechanism of the prolongation of gastric emptying time produced by anoxemia has been shown (Crisler, Van Liere and Wiles) to be on a vagospastic pylorospastic basis. After a certain threshold is passed, however, further delay is produced by a definite loss of gastric motility.

It is of interest to note that human subject number 2 showed a decrease of 12 per cent in the gastric emptying time on the third day. Comparable results were obtained in certain subjects under very moderate degrees of anoxemia. It would seem that mild degrees of anoxemia can actually stimulate gastric motility.

The results indicate that patients who have suffered a considerable loss of blood should be given food which is easily digested and which will leave the stomach rapidly. Mention must be made of the fact that a loss of blood of 500 cc. in man cannot be regarded as a grave hemorrhage. Blood donors are often called upon to give this amount and more. The subjects, moreover, reported in this paper were not exposed to shock. It is quite possible that the delay in the gastric emptying time would be much greater if the subject suffered from shock and hemorrhage both.

SUMMARY AND CONCLUSIONS

The normal gastric emptying time was determined fluoroscopically in four healthy male subjects. After one-tenth of their calculated blood volume was withdrawn (about 500 cc. in a 68 K. man), the gastric emptying time was prolonged an average of 41 per cent. In no case was it less than 25 per cent. Twenty-four hours after the hemorrhage three of the four men still showed a delay from 15 to 20 per cent. One subject showed no delay 24 hours after the blood had been withdrawn. At the end of 48 hours the stomach had apparently regained its normal motility in all the subjects.

Observations were also made on two dogs which had had one-tenth of their calculated blood volume withdrawn. The dogs too showed a distinct prolongation in gastric emptying time confirming the results found in man.

The authors wish to express their sincere thanks to Dr. C. B. Pride for withdrawing the blood from the human subjects.

REFERENCES

- CRISLER, G., E. J. VAN LIERE AND I. A. WILES. *Am. J. Digest. Dis. and Nutrition* 2: 221, 1935.
VAN LIERE, E. J., G. CRISLER AND D. ROBINSON. *Arch. Int. Med.* 51: 796, 1933.
VAN LIERE, E. J., D. H. LOUGH AND C. K. SLEETH. *Arch. Int. Med.* 58: 130, 1936.
VAN LIERE, E. J., G. CRISLER AND I. A. WILES. *J. Lab. Clin. Med.* In press.

TOTAL PLASMAPHERESIS

JOHN B. STANBURY, EDNA WARWEG AND WILLIAM R. AMBERSON

WITH THE TECHNICAL ASSISTANCE OF VERDA I. McLENDON

From the Department of Physiology, College of Medicine, University of Tennessee, Memphis, and the Marine Biological Laboratory, Woods Hole, Mass.

Received for publication June 4, 1936

Since Abel, Rowntree and Turner (1914) introduced the method of plasmapheresis the technic has been widely used to study the physiological consequences of partial plasma removal. Usually the washed cells have been re-suspended in colloid-free Ringer-Locke solutions, and re-injected. Using this method Smith, Belt and Whipple (1920), Whipple, Smith and Belt (1920) and Belt, Smith and Whipple (1920) always observed fatal shock in dogs when the plasma proteins were reduced below 1 per cent. They concluded that "blood serum proteins are stabilizing or protective factors" and that "physiological perfusion of organs is very difficult, and slight modification of the blood plasma may have a profound effect upon body cells." Drinker (1927) observed capillary dilatation and edema in the web of the frog perfused with Ringer solution containing 3 per cent gum acacia. Upon the addition of as little as 15 per cent horse serum these phenomena disappeared. He concluded that "serum possesses a peculiar power of restraining capillary dilatation and . . . leakage." Krogh (1929) similarly concludes that "there is evidently something in . . . serum, apart from its colloidal properties, which is essential to keep the capillaries of the frog's web in a normal state of permeability. . . . The power may be inherent in the serum proteins or it may be a special hormone." Drinker and Field (1933) state that probably "in acute plasmapheresis capillaries all over the body experience change, becoming temporarily more permeable to protein."

It is, however, certain that many cells and tissues in the body are not thus dependent upon the presence of proteins in their fluid environment. Such fluids as aqueous humor, glomerular filtrate, and cerebro-spinal fluid are normally nearly, or quite, protein free. Tissue fluid is believed by many to have a very low protein content (Krogh, Landis and Turner, 1932; Landis, Jonas, Angevine and Erb, 1932; Peters, 1935). However others consider that higher values, approximating those in lymph, are normally present in tissue fluid (Drinker and Field, 1933).

Our own interest in the problem of the functional significance of the

plasma proteins was first aroused in connection with our work with hemoglobin-Ringer (Amberson, Flexner et al., 1934), in the course of which we observed that all but the last traces of the normal blood could be washed out and replaced by this solution without evidence of shock. It was obvious that, under our experimental conditions, the normal plasma proteins must be reduced to the vanishing point, and we stated that "either hemoglobin in solution is able effectively to replace the proteins . . . in their control of normal function, or the ill effects ascribed to their diminution or absence have been incorrectly interpreted, and caused more by changes in the physical properties of the blood than by any specific chemical influence." Since hemoglobin is also a protein we have now completely removed the blood plasma by using solutions containing gum acacia, a colloidal polysaccharide.

METHOD. From three or four animals (cats or dogs) we collect sufficient blood to furnish cells for the experiment. The serum is removed from the defibrinated blood, and the cells are three times washed through Ringer-Locke. A total plasmapheresis requires 300 to 400 cc. of solution per kilo of body weight. The washed cells are mixed with Ringer-Locke containing 6 per cent gum, in the proportion of 30 to 40 per cent cells to 70 to 60 per cent gum-saline. In adding the salts we omit the CaCl_2 since the acacia contains enough Ca to balance the solution. We use 200 mgm. per cent glucose and 20 mgm. per cent NaHCO_3 .

The mixture of cells with gum-saline must not be made until the very moment of the infusion. Gum causes a very rapid sedimentation of the red cells (Lucia and Brown, 1934). If the mixture is allowed to stand some of the cells form clumps which cannot be broken up even by vigorous shaking. These act as emboli and may give rise to a variety of central nervous disorders.

The blood is removed under a light ether anesthesia. The carotid artery is brought through the skin by way of a small incision, and cannulated. We find it convenient to use a water-jacketed cannula whose temperature is held at about 45°C . by passing hot water through the outer jacket. The animal is first bled through a side tube and the blood volume is then restored by infusing an equal volume of red-cell-gum-saline through the main tube of the cannula. In cats we remove and inject 50 cc. at a time; in dogs we use 100 cc. Infusion is backward toward the heart under pressure.

In dogs, after plasmapheresis, further blood samples are readily secured by venous puncture; we therefore close the neck wound at once. Several weeks later it is possible to cannulate the same artery again and repeat the operation. In cats blood sampling is more difficult. We usually leave a small glass cannula in place in the carotid and find it possible to keep the wound reasonably clean by the application of bandages soaked in Dakin's fluid, or other antiseptic solution. Samples are drawn through this

cannula for the first three days. After this time it is usually necessary to recannulate farther down on the same vessel. We have not attempted to keep cats for extended periods, killing them after six to nine days. Dogs, however, are easy to keep indefinitely, even after two successive plasma removals.

Plasma proteins have been determined by the Kjeldahl method after fractionation as prescribed by Peters and Van Slyke (1932). In a few experiments acacia has been determined. Values accurate to 10 per cent are secured by removing the proteins by trichloroacetic acid precipitation, followed by alcoholic precipitation of the acacia. In the presence of 70 per cent alcohol acacia precipitates slowly and may later be collected in a Gooch crucible and weighed. Such alcoholic precipitation was suggested (but apparently not used) by Keith, Power and Wakefield (1935).

RESULTS. It is not possible, by our method, to reduce the protein concentration in the circulating blood to absolute zero. All of the original blood plasma may be washed away, but it is impossible to remove all of the tissue fluid and lymph, so that proteins drain back into the blood stream from extra-vascular regions. By repeated bleedings and infusions the plasma proteins may be reduced to a level of 0.05 to 0.15 per cent; in addition there is always present a trace of vegetable protein from the gum (0.1 per cent in the 6 per cent solution).

The recovery of the animal can hardly be distinguished from that of a normal one after a similar period of etherization. We have had animals recover so quickly that they took food within two hours. Edema is never seen in cats, and only rarely in dogs. In the latter it never appears in the first day, when the proteins are at their lowest value. The internal organs of animals killed within an hour or two after the plasma removal show no pathological condition. When edema does sometimes appear in dogs, in the second, third or fourth day, it is a transient phenomenon, localized in the neck region.

A maintained blood pressure furnishes the most striking evidence that a very low plasma protein concentration is not necessarily accompanied by shock or capillary dilatation. In cats the blood pressure usually drops somewhat during the course of the plasmapheresis, but it recovers quickly, and may even rise somewhat above the original normal value. In dogs the blood pressure maintains itself with remarkable constancy, falling, of course, with each bleeding, but rising back to normal as the blood volume is restored by the next infusion. An example of such constancy in the blood pressure is shown in figure 1. In this experiment the total plasma protein fell from an original value of 6.29 per cent to 0.09 per cent at the time of the last graphic record. The terminal blood pressure is almost exactly the same as before the plasmapheresis. It is certain that no condition even remotely resembling shock could have been present at this time.

Rectal temperatures invariably decline during the course of the plasmapheresis, but the fall is not greater than that observed in normal animals after etherization for similar periods. Temperature recovery is prompt.

Rate of disappearance of gum acacia. When relatively small amounts are injected gum acacia is known to disappear fairly rapidly from the blood stream (Meek and Gasser, 1918; Huffman, 1929; Peoples and Phatak, 1935). After our massive injections acacia leaves the blood stream more slowly, possibly because the liver and other tissues, now known to act as storehouses for acacia (Andersch and Gibson, 1934), become completely filled. Thus in one typical experiment we found that, at the end of 22 hours, the acacia had fallen to 68 per cent of that originally present. At 44 hours it was 54 per cent; at 93 hours, 32 per cent; at 162 hours, 26 per cent; and at 334 hours, 4 per cent.

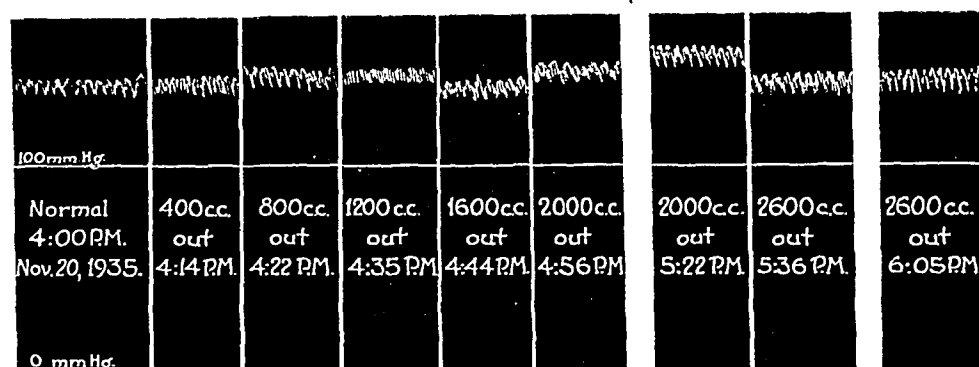


Fig. 1. Constancy of the blood pressure in a dog as its normal blood is removed and replaced by dog red-cell-gum-saline solution. In the course of the plasmapheresis the plasma proteins are reduced from 6.29 per cent to 0.06 per cent. After the second pause the proteins rise to 0.09 per cent. A trace of vegetable protein from the gum is also present.

Rate of replacement of the plasma proteins. In our preparations it is possible to follow the return of the plasma proteins from a nearly zero level. In figure 2 we show the rate of replacement in four dogs after total plasmapheresis. The various protein fractions are shown as well as the total. Replacement is complete in about 200 hours. The fibrinogen returns rapidly, whereas the albumin and globulin rise more slowly. Throughout the recovery the albumin-globulin ratio approximates that found in the original blood.

During this recovery the animals are fed, first milk on the first day, then bread and milk for several days, finally a mixed diet ad lib. We recognize the importance of diet in determining the rate of plasma protein replacement (Pommerenke, Slavin, Kariher and Whipple, 1935), but have not been immediately interested in studying this influence. Our diets have contained sufficient protein of high potency to give us a rapid rate of replacement. The data are submitted as evidence for the ability of the animals to reconstitute a normal blood after total plasmapheresis, rather than

as studies of the replacement rate per se. We believe that this method may furnish a new approach to the study of dietary factors in plasma protein regeneration.

We have kept some of our dogs for as long as three months after the operation. They are entirely normal in every respect. They also survive after a second complete removal of the blood plasma two or three weeks after the first plasmapheresis. The rate of replacement of the proteins, after a second removal, is identical with that observed after the first. We have seen no evidence of the effect described by Dick, Warweg, and Andersch (1935) who found that repeated injections of gum-saline acted drastically to reduce the plasma proteins. Our experimental conditions are rather different from theirs.

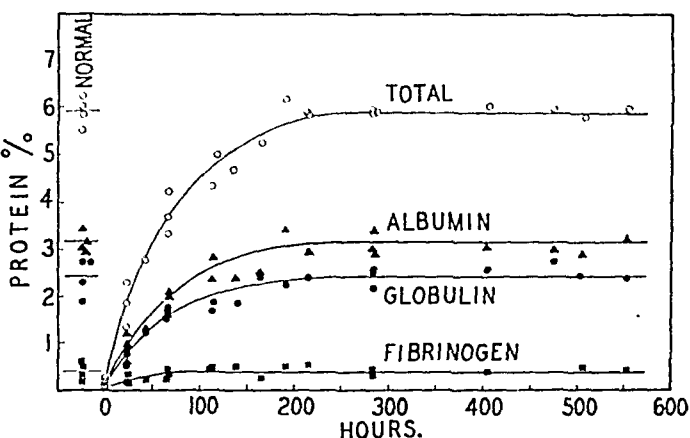


Fig. 2

Fig. 2. Replacement of plasma proteins in four dogs after total plasmapheresis.

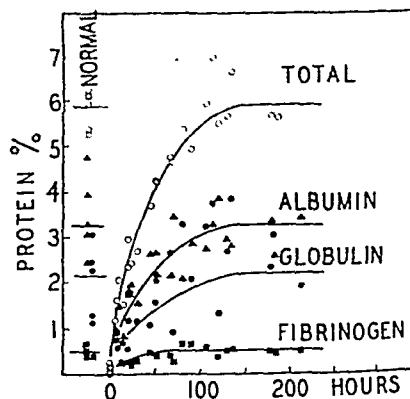


Fig. 3

Fig. 3. Replacement of plasma proteins in six cats after total plasmapheresis.

In figure 3 we show the rate of replacement of plasma proteins in six cats after total plasmapheresis. The rate is definitely more rapid than in dogs. Normal values are reached in about 125 hours. The albumin-globulin ratio is highly variable during the recovery period, but the total protein returns at practically the same rate in all animals.

DISCUSSION. It is clear that the mammalian body can survive after the removal from the blood of all but the last traces of the plasma proteins. For short periods, at least, they may be replaced, not only by the protein, hemoglobin, as in our earlier experiments, but by the colloidal polysaccharide, gum acacia. Unfortunately for the strength of this demonstration a very low plasma protein cannot be maintained for long. There is evidence (Holman, Mahoney and Whipple, 1934, and others) that a plasma protein reserve exists in the mammalian body. There is also a considerable quantity of protein in tissue fluid and lymph, presumably separate from the true reserve. From several sources, therefore, protein

is quickly mobilized. Thus in five dogs (of another series than that given above) in which total protein was originally 5.77 per cent, on the average, the value fell to 0.08 per cent after total plasmapheresis, but rose to 1.01 per cent four hours later.

We consider that, in many plasmapheresis investigations, the observations have been vitiated by a failure to maintain an adequate oxygen supply to the various tissues of the body. Certainly the original method of Abel, Rowntree, and Turner (1914), in which the washed cells are returned to the body suspended in colloid-free Ringer-Locke, must inevitably lead to tissue edema and diminished blood volume, with partial asphyxiation as an immediate consequence. Lack of oxygen is known to exercise the most powerful influence upon capillary permeability (Landis, 1928). In the perfused mammalian heart Ort, Power and Markowitz (1931) and Ort and Markowitz (1931) have found that, in the absence of red cells, gum saline is unable to maintain the beat, with much gum leaving the blood stream and penetrating into the tissue, whereas, when sufficient cells are present, little or no gum leaves the vessels, and the heart beats for some hours. Since gum actually diminishes the oxygen capacity of the whole blood (Christie, Phatak and Olney, 1935) the danger of insufficient red cells, or of reduced blood volume, becomes even more evident.

From our observations we conclude that the walls of the blood vessels, and the tissue cells generally, are by no means so sensitive to low protein in the plasma as many workers have believed. The plasma proteins undoubtedly give certain essential physical properties to blood plasma, but other colloids, even colloidal polysaccharides, can take their place. It is still conceivable that there may be a capillary hormone controlling permeability, but these results make it highly improbable that the plasma proteins are responsible. We consider our results to support the view that these proteins have mainly a physical significance, giving a proper viscosity and colloidal osmotic pressure to the blood and thereby regulating its flow and volume.

SUMMARY

The blood plasma may be almost totally removed from the blood vessels of cats and dogs, without injury to the animals, if the normal blood is replaced by a solution of 6 per cent gum acacia in Ringer-Locke, in which are suspended washed red cells of the same species (30 to 40 per cent).

This substitution is done by alternate bleeding from, and injection into, the carotid artery, until a large volume of the solution, of the order of 300 to 400 cc. per kilo, has been passed through the body. The plasma proteins may thus be reduced to the very low level of 0.05 to 0.15 per cent.

No signs of shock, or of capillary dilatation and edema, appear after such total removal of the plasma. The blood pressure is maintained, and the animals recover quickly and may live indefinitely.

When an adequate diet is given the plasma proteins return to normal in about 200 hours in dogs, and 125 hours in cats.

It is concluded that the plasma proteins are not specifically necessary for the maintenance of normal function. Other colloids, even polysaccharides such as gum acacia, may be substituted for them, if these are able to maintain blood volume, and if sufficient oxygen is present in the solution. The major function of the plasma proteins is physical, in regulating blood flow and volume, by maintaining the viscosity and colloidal osmotic pressure of the plasma.

It is a pleasure to acknowledge the support given to this work by the American Philosophical Society, from the Penrose Fund, and by the Council on Pharmacy and Chemistry of the American Medical Association. We are also indebted to the Eli Lilly Company, of Indianapolis, for supplying the acacia.

REFERENCES

- ABEL, J. J., L. G. ROWNTREE AND B. B. TURNER. *J. Pharmacol. Exper. Therap.* **5**: 625, 1914.
- AMBERSON, W. R., J. FLEXNER, F. R. STEGGERDA, A. G. MULDER, M. J. TENDLER, D. S. PANKRATZ AND E. P. LAUG. *J. Cell. and Comp. Physiol.* **5**: 359, 1934.
- ANDERSCH, M. AND R. B. GIBSON. *J. Pharmacol. Exper. Therap.* **52**: 390, 1934.
- BELT, A. E., H. P. SMITH AND G. H. WHIPPLE. *This Journal* **52**: 101, 1920.
- CHRISTIE, A., N. M. PHATAK AND M. B. OLNEY. *Proc. Soc. Exper. Biol. and Med.* **32**: 670, 1935.
- DICK, M., E. WARWEG AND M. ANDERSCH, *J. A. M. A.* **105**: 654, 1935.
- DRINKER, C. K. *J. Physiol.* **63**: 249, 1927.
- DRINKER, C. K. AND M. E. FIELD. *Lymphatics, lymph and tissue fluid.* Williams & Wilkins, Baltimore, 1933.
- HOLMAN, R. L., E. B. MAHONEY AND G. H. WHIPPLE. *J. Exper. Med.* **59**: 251, 1934.
- HUFFMAN, L. D. *J. A. M. A.* **93**: 1698, 1929.
- KEITH, N. M., M. H. POWER AND E. G. WAKEFIELD. *Proc. Staff Meetings, Mayo Clinic* **10**: 38, 1935.
- KROGH, A. *The anatomy and physiology of capillaries.* Yale Univ. Press, New Haven, 1929.
- KROGH, A., E. M. LANDIS AND A. H. TURNER. *J. Clin. Invest.* **11**: 63, 1932.
- LANDIS, E. M. *This Journal* **83**: 528, 1928.
- LANDIS, E. M., L. JONAS, M. ANGEVINE AND W. ERB. *J. Clin. Invest.* **11**: 717, 1932.
- LUCIA, S. P. AND J. W. BROWN. *Proc. Soc. Exper. Biol. and Med.* **32**: 189, 1934.
- MEEK, W. J. AND H. S. GASSER. *This Journal* **47**: 302, 1918.
- ORT, J. M. AND J. MARKOWITZ. *This Journal* **96**: 541, 1931.
- ORT, J. M., M. H. POWER AND J. MARKOWITZ. *This Journal* **98**: 163, 1931.
- PEOPLES, S. A. AND N. M. PHATAK. *Proc. Soc. Exper. Biol. and Med.* **32**: 635, 1935.
- PETERS, J. P. *Body water.* C. C. Thomas. Springfield, 1935.
- PETERS, J. P. AND D. D. VAN SLYKE. *Quantitative clinical chemistry.* Vol. II. Methods. Williams & Wilkins, Baltimore, 1932.
- POMMERENKE, W. T., H. B. SLAVIN, D. H. KARIHER AND G. H. WHIPPLE. *J. Exper. Med.* **61**: 261, 283, 1935.
- SMITH, H. P., A. E. BELT AND G. H. WHIPPLE. *This Journal* **52**: 54, 1920.
- WHIPPLE, G. H., H. P. SMITH AND A. E. BELT. *This Journal* **52**: 72, 1920.

THE ACTIVITY OF THE CARDIAC SYMPATHETIC CENTERS

D. W. BRONK, L. K. FERGUSON,¹ R. MARGARIA² AND D. Y. SOLANDT³

*From The Eldridge Reeves Johnson Foundation for Medical Physics,
University of Pennsylvania*

Received for publication June 8, 1936

The development of methods for recording the nerve impulses going to and from the centers provides an effective means for studying the activity of the sympathetic nervous system (Adrian, Bronk and Phillips, 1932). Such a direct analysis of the behavior of the sympathetic nerve cells is especially valuable because it is difficult to determine their functional characteristics from the response of the organs which they supply. The inertia of these effectors is so great that the activity produced by the individual nerve impulses is masked or obscured, a situation which is quite different from that in the somatic system where a study of the contraction in striated muscle has given much information regarding the properties of the individual motor nerve cells.

In the present investigation we have undertaken to determine the character of the sympathetic nerve discharge to the heart. The purpose of the experiments has been to add to our knowledge concerning the activity of the sympathetic nervous system and to give a clearer understanding of the nature of cardiac control.

METHODS AND PROCEDURES. Most of the experiments were performed on cats under light nembutal anesthesia but in a few cases decerebrate animals were employed without significantly different results. In every case the animal was kept in a humid chamber at a temperature of about 34°C. The chest wall was removed from the first to the sixth ribs, after which respiration was maintained by means of a pump. It was then possible to reach the long cardiac-sympathetic nerves which run from the stellate ganglia to the inferior cardiac plexus and contain both chronotropic and inotropic fibers. One of these nerves was dissected free from the surrounding tissues and transected some distance from the heart. The proximal portion was then slung onto silver-silver-chloride brush electrodes leading to a direct-coupled or resistance-capacity-coupled amplifier and

¹ Associate in Surgery.

² Fellow of the Rockefeller Foundation.

³ Research Associate, University of Toronto.

Matthews oscillograph. In this way the efferent sympathetic impulses in the cardiac nerves were recorded.

RESULTS. *The tonic discharge.* Records of the sympathetic discharge to the heart are shown in figure 1. In all our experiments on 46 animals we have found such continuous activity in the cardiac sympathetic nerves, provided the animal was in reasonably good condition. This statement is not to be construed, however, as meaning that the degree of that activity is constant. It is indeed increased by asphyxia, decreased by overventilation or a rise in blood pressure, and largely modified by numerous other factors. But in general, under the conditions of our experiments in which the blood pressure and depth of respiration were permitted to vary within wide limits, there was a persistent discharge of impulses. This is positive evidence, then, in support of the view held by Hunt (1899) and others that the heart is under a continual influence of sympathetic impulses.

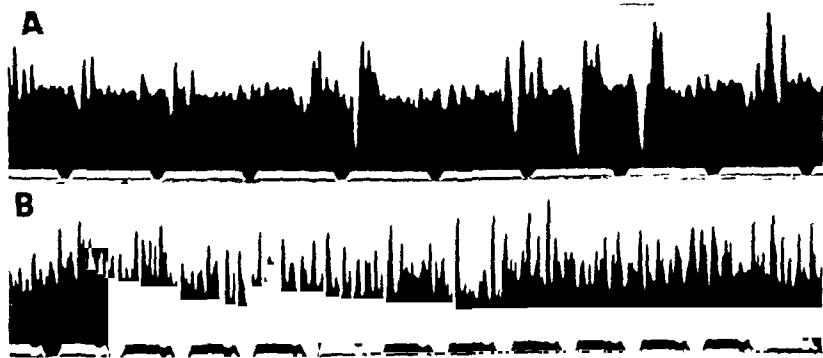


Fig. 1. Records showing ungrouped "tonic" discharge of impulses in a sympathetic cardiac nerve from the stellate ganglion. A, in relatively few fibers; B, in the whole nerve. Time marker in this and subsequent records gives $\frac{1}{3}$ second intervals.

The fiber pathways. The impulses shown in figure 1 and elsewhere in this paper have been recorded in postganglionic fibers for they are abolished by painting the stellate ganglion with nicotine. The pathways of the pre-ganglionic impulses by which they are initiated have been determined by cutting successively the first to the fifth thoracic rami. When the first or second thoracic ramus is severed there is little decrease in the magnitude of the postganglionic discharge. Section of either the third or fourth ramus, on the other hand, produces a very considerable decrease, and when they are both cut practically all the impulses are eliminated. Our experiments show therefore that most of the sympathetic impulses going to the heart from the stellate ganglion enter by way of the third and fourth thoracic rami.

The course of many of the postganglionic fibers is in one or more nerves running from each ganglion to the cardiac plexus. A considerable number

of the fibers, however, go directly from the ganglia to the vagi and run with those nerves toward the heart so that it is always possible to observe a discharge of sympathetic impulses in the vagus nerves.

It is of course not possible in our experiments to restrict the fibers under observation to those concerned with the control of the rate and force of the cardiac beat. But anatomical evidence indicates that the great majority of the efferent fibers in these nerves going to the cardiac plexus from the stellate ganglia have that function. It is fair to assume therefore that our results give typical evidence concerning the activity of the sympathetic nerve cells regulating the heart.

The source of the potentials. The type of action potential with which we are dealing is illustrated in figure 2A. A maximum electric stimulus was applied to a postganglionic nerve from the stellate ganglion and the response recorded after an interval which indicates that the impulses are

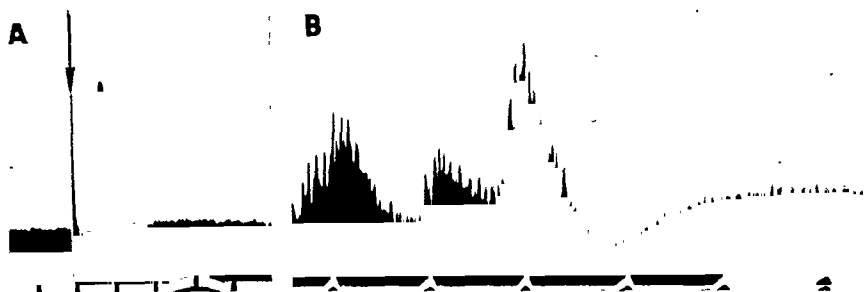


Fig. 2. A. Action potential in a postganglionic nerve from the stellate ganglion elicited by a single shock to nerve (indicated by arrow). Time: 0.025 second. B. Positive after potential revealed when the normal efferent discharge in the postganglionic fibers was suddenly inhibited reflexly by a rise in blood pressure in aorta and carotid sinuses produced by clamping the descending aorta.

transmitted with a velocity which varies from 0.6 to 1.5 m. per second in different fibers. They are characterized by large positive after potentials which become especially apparent at the end of a period of sustained stimulation or whenever the normal efferent discharge is suddenly inhibited reflexly as in figure 2B.

The individual potential pulses in such efferent discharges as are shown in figure 1 are of considerable magnitude and may be as great as 50 microvolts. They are therefore too large to be the action potentials in single postganglionic fibers. In discussing similar large potential pulses in the hypogastric and cervical sympathetic nerves Adrian, Bronk and Phillips (1932) suggested that they are produced by many postganglionic fibers functioning as a unit and in fairly close synchronism. This is because one preganglionic fiber activates many postganglionic fibers (Billingsley and Ranson, 1918). Thus the ganglion functions as a natural amplifier.

The random and irregular character of the discharge in figure 1 indicates that we are dealing with more than one such group of postganglionic fibers innervated by a single preganglionic fiber. We have frequently tried to observe the impulses in a single unit by cutting and splitting the trunk but the small size of the fibers and the fragile character of the nerve make this a difficult task. One can more readily record the impulses in a single preganglionic fiber, and that is just as useful in analyzing the activity of the individual sympathetic motor nerve cell. In such single preganglionic fibers we have observed impulse frequencies as low as one or two per second and seldom higher than ten or twenty a second. This is in marked contrast with the activity of somatic motor nerve cells from which the rate of impulse discharge may be five or ten times as great (Adrian and Bronk, 1928). Such differences are interesting in view of the slower characteristics and greater inertia of the organs supplied by the sympathetic nervous system. Mammalian skeletal muscle fibers require 30 to 350 impulses a second in order that they may develop their maximum tension (Cooper and Eccles, 1930), whereas sympathetic stimulation of 10 to 15 a second produces maximum cardiac acceleration (Bronk and Pumphrey, 1935). Bozler (1936) has also found that low frequencies of sympathetic stimulation (2-3 a second for the frog) are sufficient to maintain the blood vessels in a state of maximal constriction, and only when the frequency of sympathetic stimulation is low are dorsal root impulses effective in modifying the degree of contraction.

Grouped impulses. One of the most striking characteristics of the nervous discharge in the cardiac sympathetic fibers is the frequent grouping of impulses to form waves which may be several hundred microvolts in amplitude. Because of their size and their form they almost certainly represent the activity in many more postganglionic fibers than are supplied by a single preganglionic fiber. These waves are therefore not due to the type of ganglionic grouping referred to above. It might still be argued, however, that they are the result of some property of the ganglion whereby the activity of the various units is synchronized. Such a possibility is indeed suggested by Tower's (1933) interesting observation of wave-like groups of impulses in sympathetic fibers from the isolated viscera of a frog. She tentatively proposes that "it is not beyond possibility that the cellular plexi of the viscera or ganglia contain something analogous to a synchronizing mechanism."

It is more probable however that these volleys of impulses which we are considering may be due to a more or less synchronous rise and fall in the level of excitation of large numbers of sympathetic motor cells in the cardiac centers. That this is the correct explanation is shown by the fact that similarly grouped impulses are observed in the preganglionic nerves.

Such synchronized firing of the cells in the sympathetic centers is further

illustrated by an experiment which was designed to observe the relation between the activity on the two sides of the body. A nerve from the right stellate ganglion was placed on electrodes leading to one amplifier and oscillograph; a nerve from the left stellate ganglion was placed on electrodes leading to a second amplifier and oscillograph. In this way simultaneous records were made of the impulses from the ganglia on the two sides. A



Fig. 3. Grouped impulses in right and left sympathetic cardiac nerves recorded simultaneously with two recording systems, to show synchronization of activity on the two sides.

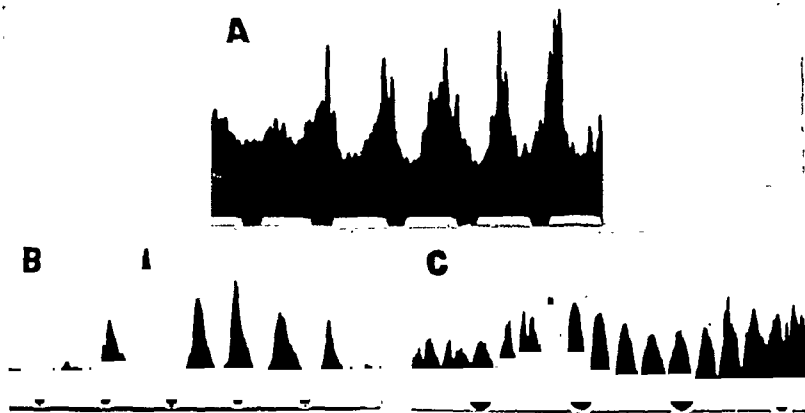


Fig. 4. Efferent sympathetic cardiac impulses grouped into regularly recurring waves. A. Record from relatively few fibers. Frequency of waves, 6 per second. B. More fibers in action, lower amplification. Frequency 7 per second. C. Frequency 20 per second.

typical record is shown in figure 3, where it will be seen that the groups of impulses from the two ganglia are synchronous. Because this rhythmic activity is bilaterally synchronous we must conclude that the groups of motor cells on the two sides are closely connected, so that they readily interact and modify each the other's activity, or are subject to a common driving influence which causes their rhythmic excitation. This is reminiscent of the findings of Gasser and Newcomer (1921) on the discharge of

motor impulses in the phrenic nerves. Recording simultaneously the impulses in the nerves on both sides they found rapidly recurring waves of activity within each respiratory cycle which occurred simultaneously in the two nerves. From such experiments they decided that "the intervals at which impulses are discharged from the cord into the nerve from the two sides are controlled by the same common point."

The groups of impulses in the cardiac nerves are sometimes discharged at irregular intervals, or at other times with marked regularity as shown by figure 4. In A the frequency of the waves is about 6, in B about 7 and in C they occur 20 times a second. These are three rhythms commonly observed in the sympathetic system; the form of the waves indicates that

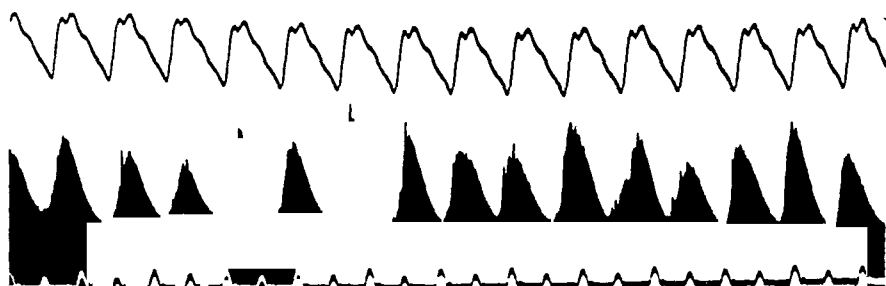


Fig. 5. Grouped discharge with the frequency of the cardiac cycle. Carotid pulse in the uppermost record.



Fig. 6. Periodic inhibition of the sympathetic discharge with each inflation of the lungs. Inflation is shown as a downward movement of the middle record (plethysmograph).

there is a periodic increase and then decrease in the number of motor nerve cells in action. This cyclical fluctuation in the activity of a large group of cells in the central nervous system, occasionally at a rate corresponding to that of the Berger rhythm, is of especial interest at the present time when considerable attention is being paid to rhythmic processes in the brain.

Such regularly recurring waves as are described in the previous paragraph do not often persist continuously for more than a few seconds at a time. A rhythm appears for a short interval, then disappears or becomes obscured by a more irregular activity, and reappears again. Two other types of rhythmically grouped discharges of lower frequency are more persistent, and the mechanism of their production is more susceptible to analysis. They are discussed in the next section.

Cardiac and respiratory groupings. Although the rhythmic volleys of impulses we have been considering are related to no other obvious rhythm of the organism we have often observed groups of impulses in phase with the heart beat or respiration. This periodic activity synchronous with the cardiac or respiratory cycles has already been found in sympathetic nerves supplying constrictor impulses to blood vessels (Adrian, Bronk and Phillips, 1932).

An instance in which the impulses are grouped into large waves, occurring with the frequency of the heart, is shown in figure 5 where synchronous with each cardiac cycle there is a large volley of efferent sympathetic impulses. The activity of the motor nerve cells is in some way inhibited or stimulated synchronous with the pulse, so that there is a rhythmic beating of the centers. The mechanism will be discussed later.

Less frequently we have observed a grouping of the impulses with respiration as shown in figure 6, the discharge occurring during expiration.

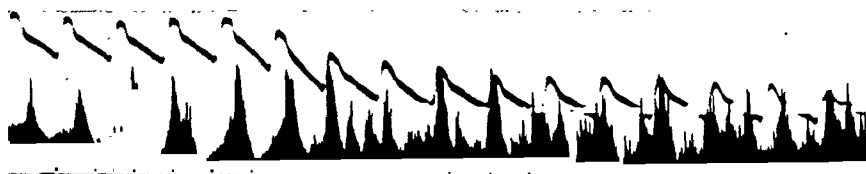


Fig. 7. Volleys of sympathetic impulses synchronous with the pulse. As pressure falls cardiac rhythm disappears and discharge becomes more continuous. Uppermost record: pressure in carotid artery.

In many animals both the cardiac and respiratory groupings are present at the same time, as in the figure presented.

It is apparent that those groups of motor nerve cells which control the rate and force of the cardiac contraction possess no one characteristic rhythm. The cells show a marked tendency to work in synchronism with a resulting grouped discharge; but the rhythm with which these volleys occur is highly variable for the sympathetic centers appear to be extremely labile and subject to various influences. Certain of these can be described.

Afferent control of sympathetic rhythms. There are two fairly obvious explanations of the bursts of sympathetic impulses which occur with the frequency of the pulse. A. The periodic pulsation of blood pressure within the central nervous system might act as a mechanical stimulus to the motor nerve cells causing their periodic excitation. B. Bronk and Kaltreider (1931), and Bronk and Stella (1932), have shown that there is a volley of afferent impulses discharged from the receptors in the arch of the aorta and the carotid sinus with each systolic rise in pressure. These rhythmically

recurring groups of impulses, playing upon the sympathetic centers, may cause them to function with a corresponding rhythm.

Our experiments show the latter to be the most important, if not the sole cause, of the cardiac grouping for section of the carotid sinus and aortic nerves usually abolishes such synchronized activity in the sympathetic nerves. A frequent observation which gives further support to this view is illustrated in figure 7. Previous to the beginning of the record the blood pressure in the upper part of the animal had been elevated by clamping the descending aorta; it will be observed that as the record begins the sympathetic cells discharge a volley of impulses with each cardiac cycle. When the pressure falls there is more continuous activity and no simple grouping of the impulses synchronous with the pulse. The lower pressure is a less effective stimulus of the receptors in the carotid sinus and aorta, and the consequent reduction in number of afferent impulses makes less effective their influence on the sympathetic centers. It seems probable, therefore, that when the excitation of the cells in the cardiac sympathetic centers rises and falls with the frequency of the heart the variation in activity is somehow due to the pulsatile volleys of afferent impulses from the walls of the blood vessels.

In rare cases there is a suggestion of a cardiac rhythm in the sympathetic discharge after both carotid sinus and aortic nerves are cut. In such cases there is the possibility that impulses from blood vessels which travel over other afferent pathways may be the responsible agents. Thus Gammon and Bronk (1935) have reported impulses from Pacinian corpuscles in the mesentery which are sometimes in volleys synchronous with the pulse, and recently such grouped afferent impulses have been observed in fibers passing through the stellate ganglion (Bronk and Larrabee, 1935).

The cessation of sympathetic impulses during inspiration has been described earlier in the paper. Such rhythmic activity appears to be due to periodic inhibition of the centers by afferent impulses from stretch receptors in the lungs, for the rhythm disappears after section of the vagi. This control of the cardiac sympathetic centers is further illustrated by experiments in which the lungs are distended to varying degrees and so maintained. On distention there is a complete inhibition of the sympathetic discharge for a period of time which depends upon the degree of inflation, and subsequently there is a gradual escape from the inhibition. Similar results are obtained by electrical stimulation of the central ends of the cut vagi.

Such a relation between the respiratory cycle and the activity of the cardiac sympathetic centers has been observed in fourteen animals; in only one have we found a discharge of impulses during inspiration and in that instance the rhythm persisted after section of the vagi. This latter observation recalls the experiments of Adrian, Bronk and Phillips (1932)

in which there was occasionally a respiratory grouping in the cervical sympathetic and hypogastric nerves, the discharge occurring during inspiration. Because the influence of respiration on sympathetic activity was greater after section of the vagi and because in the curari-immobilized animal there were bursts of sympathetic impulses synchronous with the motor discharges in the phrenic nerves it was concluded that the respiratory grouping "was due to the direct action of the respiratory center on the vaso-motor center." Our present experiments indicate that the cardiac sympathetic center may likewise be directly excited by the respiratory center but is more often under the inhibitory influence of afferent impulses from the lungs or, more often still, is free from any apparent nervous connection with respiratory activity.

Rhythmic driving of the centers. The preceding experiments indicate the importance of afferent impulses from the viscera in regulating the rhythmic

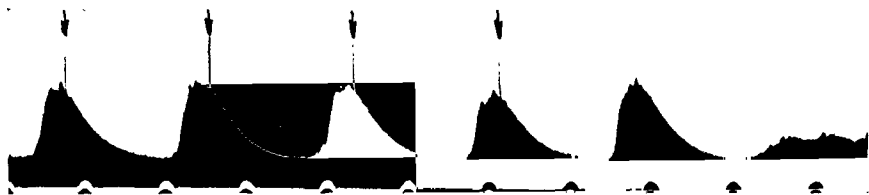


Fig. 8. Rhythmic waves of activity (grouped impulses), recorded in sympathetic cardiac nerve and reflexly driven by stimulating central end of aortic nerve at times indicated by arrows. Each wave is associated with the previous stimulus (interval between stimulus and the associated wave 0.3 sec.)

activity of the cardiac sympathetic centers. Such afferent determination of the efferent rhythm is well illustrated by the following observations.

During the course of an experiment in which we were stimulating electrically the central end of an aortic nerve we found at high stimulus frequencies inhibition of the centers and subsequent escape. But as the rate of stimulation was lowered the centers began to discharge rhythmically and with the frequency of the stimulus. This is shown in figure 8. When the frequency of afferent excitation was lower than a certain critical level the centers failed to follow, and the discharge again became random and irregular. We have repeated this on many different animals, stimulating either the aortic or carotid sinus nerves, and we frequently find that the cells comprising the cardiac sympathetic centers can be driven rhythmically at rates varying from about 2 to 15 per second. Apparently the time constants of the cells are such that they are unable to respond with synchronous and grouped discharges above and below these frequencies.

It is not improbable that the genesis of such artificially induced rhythms is similar to that of the grouped discharges synchronous with the pulse.

In both cases the mechanism would appear to be an inhibition of the sympathetic activity by the volley of afferent impulses, followed by release, and again inhibition by the next afferent volley. Thus a systolic rise in pressure initiates in the carotid sinuses and aorta bursts of afferent impulses which, arriving at the centers inhibit their activity. The discharge begins again during the latter part of diastole as in figure 5, reaches its maximum during the next systole and is again inhibited by the afferent impulses accompanying that systole. The phase of the cardiac cycle during which the impulses are observed in the sympathetic nerve will of course depend upon the conduction times for the afferent and efferent impulses and the latent period of the centers.

The sequence of events in figure 8 is probably to be accounted for in a similar manner but certain observations indicate that the complete explanation of the phenomenon may involve additional considerations. For instance, in two experiments the volleys of sympathetic impulses persisted for several seconds after the cessation of the stimuli and at the same regular frequency, a persistence which was much longer than could be accounted for by a lag or latency of the centers (only 0.3 sec. at the beginning of the stimulus). Only gradually did the centers lose the rhythm which had previously been imposed upon them by the volleys of afferent impulses. We must conclude that there are some obscure factors in the mechanism whereby the activity of the sympathetic motor cells is set into resonance with the afferent impulses.

DISCUSSION. The importance of the sympathetic nerve supply to the heart has been debated frequently and some authors have doubted whether the accelerator and augmentor fibers are normally in action. The present experiments give definite evidence as to the actual state of activity in those fibers and show that they conduct a fairly continuous series of impulses to the heart. They thus support the view of Hunt (1899) and others that the heart is under a continual sympathetic influence. It is the opinion of such writers that the efficacy of vagal impulses is largely determined by the accelerator background existing at any moment; the greater the sympathetic activity, the greater the effect produced by a given variation in vagal discharge. If such be the rôle of the cardiac sympathetic impulses, effective control of the heart would depend in considerable measure upon a ready modification of the sympathetic discharge by impulses reporting the condition of the viscera. Such a lability of the cardiac sympathetic centers under the influence of afferent impulses has been amply revealed by our experiments and is in agreement with the original observations of Hering (1894) and of Hooker (1907) which established the reflex control of the cardiac accelerator nerves.

The pronounced grouping of the efferent impulses which results from the rhythmically synchronized discharge of the motor nerve cells appears as

one of the most striking and characteristic properties of the sympathetic system. This is not without analogy in somatic nerves although there the frequency of the grouping is usually quite different. For instance, Dittler and Garten (1912) and Gasser and Newcomer (1921) have shown that in the electric response of the whole phrenic nerve there are oscillations at the rate of about 70 a second. Adrian and Bronk (1928) subsequently found that when the individual motor nerve cells are discharging at a low frequency there is no synchronization of the discharge from the several cells; not until the frequency of impulses rises to 60-70 a second does any appreciable synchronization develop. Thus a smooth contraction is maintained for if there were synchronized groups of impulses of a low frequency they would cause a gross tremor of the muscle. Here in the sympathetic nerves, however, we find the frequency of the impulse volleys varying from one every 3 or 4 seconds to about 20 per second. It is of interest to consider their effect upon cardiac activity.

The nature of the cardiac musculature and its relation to the extrinsic nerves are such that it is probably of little significance whether the impulses in the several fibers arrive synchronously or out of phase with one another. But the relative effects of volleys of impulses recurring at frequent or infrequent intervals is less obvious. In preliminary experiments (Bronk and Pumphrey, 1935) we have imitated these efferent volleys of various frequencies by electrically stimulating the peripheral end of a sympathetic cardiac nerve. When short bursts of impulses are delivered more frequently than every five seconds no rhythmic variation in the heart rate is observed. If the groups of stimuli are applied at longer intervals than that, the heart rate shows fluctuations synchronous with the periods of stimulation. From this it would appear that the only type of efferent sympathetic rhythm which could be reflected in the cardiac rate is the respiratory grouping, and that only when the rate of respiration is fairly slow.

This raises the question as to whether the periodic inhibition of the cardiac sympathetic discharge is in part responsible for sinus arrhythmia. It is generally believed (cf. Samaan, 1935) that the arrhythmia is due to variations in vagal activity for it is said to be abolished by atropine or by section of the vagi. On the other hand, our experiments show that the sympathetic discharge to the heart does frequently vary with respiration and that these variations are capable of producing changes in the heart rate.

SUMMARY

The activity of the cardiac sympathetic centers has been investigated by recording the action potentials in the cardiac nerves from the stellate ganglia of the cat.

There is a fairly continuous discharge of impulses which exert a "tonic" augmentor and accelerator influence upon the heart. This discharge is however largely modified by changes in the chemical composition of the blood and by afferent impulses.

The principal pathways of the impulses from the cord to the stellate ganglion are the third and fourth and to a lesser extent the second and fifth thoracic rami.

The impulse frequency from the individual sympathetic motor nerve cells seldom exceeds ten or fifteen a second, and is usually considerably less. This contrasts with the much higher frequency of discharge from somatic motor nerve cells.

The potential pulses in the postganglionic nerves are of considerable magnitude because of the grouping of impulses which results from the innervation of many postganglionic fibers by a single preganglionic fiber.

There are also much larger potential waves caused by the synchronous activity in very many nerve fibers. It is shown that this is due to the coordinated and rhythmic discharge from large numbers of nerve cells in the centers. This activity is bilaterally synchronous.

The grouped activity is of four types. The volleys may come at irregular intervals or at other times periodically with frequencies varying from 5 to 20 a second but unrelated to any other obvious rhythm of the organism. Or, on the other hand, the bursts of impulses may be synchronous with the pulse or the respiratory cycle.

The latter two forms of rhythmic cellular activity are largely due to afferent impulses from the viscera: bursts of impulses from the blood vessels initiated by the systolic rise in pressure or the impulses from distention receptors in the lungs.

An example of the marked effect of such afferent impulses upon the activity of the sympathetic centers is found in the observation that it is possible to drive those centers by repetitive stimulation of the central ends of the carotid sinus or aortic nerves, thus causing the motor nerve cells to discharge periodically with the frequency of the afferent impulses. This can be done within a limited range of stimulus frequencies.

The characteristically grouped discharges from the cardiac sympathetic centers cause periodic variations in heart rate only if the bursts of efferent impulses are separated by some seconds. This is due to the inertia of the effector mechanism.

The expenses of this investigation were defrayed in part by a grant from the Committee on Scientific Research of the American Medical Association.

REFERENCES

- ADRIAN, E. D., D. W. BRONK AND G. PHILLIPS. *J. Physiol.* 74: 115, 1932.
ADRIAN, E. D. AND D. W. BRONK. *J. Physiol.* 66: 81, 1928.

- BILLINGSLEY, P. R. AND S. W. RANSOM. *J. Comp. Neurol.* **29**: 367, 1918.
- BOZLER, E. *This Journal*, in press.
- BRONK, D. W. AND R. J. PUMPHREY. Unpublished observations.
- BRONK, D. W. AND N. C. KALTREIDER. *This Journal* **97**: 508, 1931.
- BRONK, D. W. AND G. STELLA. *J. Cell. and Comp. Physiol.* **1**: 113, 1932.
- BRONK, D. W. AND M. G. LARRABEE. Unpublished observations.
- COOPER, S. AND J. C. ECCLES. *J. Physiol.* **69**: 377, 1930.
- DITTLER, R. AND S. GARTEN. *Ztschr. f. Biol.* **58**: 420, 1912.
- GAMMON, G. D. AND D. W. BRONK. *This Journal* **114**: 77, 1935.
- GASSER, H. S. AND H. S. NEWCOMER. *This Journal* **57**: 1, 1921.
- HERING, H. E. *Centralbl. f. Physiol.* **8**: 75, 1894.
- HOOKE, D. R. *This Journal* **19**: 417, 1907.
- HUNT, R. *This Journal* **2**: 395, 1899.
- SAMAAN, A. *J. Physiol.* **83**: 332, 1935.
- TOWER, S. S. *J. Physiol.* **78**: 225, 1933.

A POSSIBLE RÔLE OF THE EOSINOPHIL LEUCOCYTES IN THE ENDOCRINE COMPLEX OF THE FEMALE RAT¹

C. P. KRAATZ

From the Department of Zoölogy, University of Cincinnati

Received for publication June 15, 1936

The experimental production of pseudopregnancy in white rats has been accomplished in two general ways: a, single stimulation of the cervix of the uterus, this, by initiating events leading to follicular inhibition and luteal activity, producing the typical early gestational picture in the uterus; b, repeated hormone administration, effecting the ovarian and uterine changes presumably by a more direct action.

Goat anti-rat thymus serum, non-specific and generally hematotoxic (Goldman and Kraatz, unpublished), injected intraperitoneally into 14 adult virgin female rats in doses of $\frac{1}{4}$ to $\frac{1}{2}$ cc. daily, resulted in 10 pseudopregnancies. The uteri of three of these pseudopregnant females were traumatized by the insertion of threads and responded with formation of macroscopic placentomata. All rats receiving doses of $\frac{1}{2}$ cc. and the smaller rats receiving $\frac{1}{4}$ cc. doses became pseudopregnant after having one or two normal cycles; while daily injections of 1 cc. of normal goat serum continued for three weeks did not affect the cycles.

The possibility that the gonadotropic hormone content of the immune serum was responsible for the effects described is doubtful since it was found that the serum had no influence on the ovaries or the maturing of young females. Furthermore, single intraperitoneal injections in 47 animals of $1\frac{1}{2}$ to 3 cc., given at prooestrus or early oestrus, resulted in 27 pseudopregnancies, nine being verified by securing positive decidual reactions. The failures may be attributed to the fact that this is apparently a "threshold reaction." Successive bleedings of the goat produced sera of varying titer and, where $1\frac{1}{2}$ cc. of a given lot would fail to evoke pseudopregnancy, 2 cc. of the same lot would succeed. The pseudopregnancies uninterrupted by autopsy ranged from 12 to 17 days in length with an average of 13.81 days. Neither normal goat serum nor goat anti-rat liver serum in single injections of 3 cc. affected the cycles. Occasionally, slight weight losses were noted following injections, the maximum being 4.9 per cent of body

¹ This paper is a thesis submitted by C. P. Kraatz to the faculty of the Graduate School of the University of Cincinnati in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Zoölogy, June, 1936.

weight in a rat injected daily, but recovery of initial weight was always completed after five days from the first injection and the animals gained from that point.

Similar doses of the anti-thymus serum in late oestrus or postoeustrus lengthened the following dioestrous period to 3 or 4 days, while single injections during dioestrus produced a continued full oestrus of 3 or more days in 8 out of 14 animals. The ovaries of 2 of these when sectioned showed follicular cysts.

Thus, an anti-thymus serum, primarily affecting blood cells in the adult rat, was found to produce a heavily luteinized ovary by a single injection during prooeustrus, and, in some cases, a cystic ovary by a single injection in dioestrus. It appeared probable that the prooeustrous injections initiated a chain of events comparable to those producing normal pseudopregnancy.

The most constant effect of serum treatment was eosinophilia. Animals injected daily were found to have eosinophil leucocytes in the circulation during pseudopregnancy ranging from 6.5 to 11.5 per cent of the total white count, while controls receiving larger amounts of normal serum for the same length of time had 3 per cent or less. Single injections resulted in a slight eosinophilia within 24 hours, the conditions becoming pronounced in 48 to 72 hours and persisting for at least 4 days (table 1 and fig. 1).

The only generally accepted belief regarding the eosinophil leucocytes is their proliferative response to foreign proteins in the circulation. Daily leucocyte counts were made on female rats of various ages and stages of sexual activity. Differential counts of 500 white cells each (1000 in immature animals) were made in a longitudinal direction on Wright-stained smears. The product of the eosinophil percentage and the white cell count for that day resulted in a figure representing the number of eosinophil leucocytes per cubic millimeter of blood. Averages of these figures, based on differential counts of 127,500 cells, are used in table 1 and figure 1.

Only the counts for regular 4-day oestrous cycles are included in the figures for normal adult females in table 1. Additional counts of slightly aberrant cycles showed the lowest eosinophil number sometimes before prooeustrus in the case of an abbreviated oestrus, or after prooeustrus in the case of lengthened oestrus, but a rise always occurred following oestrus. Individual animals maintained levels of eosinophil fluctuation relatively constant in successive cycles, but differing from the levels of other individuals. In general, the level rises with increase in age. Thus, the rats in the pseudopregnancy determinations, being young, showed lower levels in the normal cycles than the average.

Excepting the constant oestrous condition, the significant differences in the eosinophil number under various conditions parallel the probable

TABLE 1

CONDITION	SMEAR (VAGINAL)	EOSINOPHIL PER CENT	EOSINOPHILS PER C.M.M.
Normal cycles (21 cycles in 15 rats)	Prooestrus	1.705	257 \pm 31.8
	Oestrus	2.015	307
	Postoestrus	2.5	432 \pm 28.2
	Dioestrus	1.88	305
Normal pseudopregnancy with preceding cycle (7 rats—see Note)	Prooestrus	0.95	125
	Oestrus	1.34	206
	Postoestrus	1.6	233
	Dioestrus	1.64	270
	Prooestrus (Sterile copula- tion)	1.5	188
	Oestrus	2.26	373
	Pseudopregnancy (2)	2.34	346
	Pseudopregnancy (3)	2.16	457
	Pseudopregnancy (4)	1.7	285
	Pseudopregnancy (5)	2.4	306
Serum pseudopregnancy with preceding cycle (4 rats—see Note)	Prooestrus	0.9	127
	Oestrus	1.73	266
	Postoestrus	1.8	423
	Dioestrus	1.86	352
	Prooestrus (Injection)	1.5	197
	Oestrus	1.775	217
	Pseudopregnancy (2)	3.55	755
	Pseudopregnancy (3)	4.0	841
	Pseudopregnancy (4)	2.7	822
	Pseudopregnancy (5)	3.3	786
Immature females (10)	None	0.59	59 \pm 6.6
Adults with cystic ovaries (3 rats—7 days each)	Oestrus (constant)	4.41	666 \pm 44.4
50-day old littermates 2 mature—5 days each 2 immature—5 days each	Cycles	1.16	240 \pm 26.6
	None (closed)	0.18	45 \pm 9.7
<i>Normal cycles:</i> Difference between prooestrus and postoestrus.....			175 \pm 45.1
<i>Normal pseudopregnancy:</i> Average of 5 days before stimulation.....			200 \pm 15.3
Average of 5 days after stimulation.....			347 \pm 17.6
Difference.....			147 \pm 23.3
<i>Serum pseudopregnancy:</i> Average of 5 days before injection.....			305 \pm 25.4
Average of 5 days after injection.....			705 \pm 21
Difference.....			400 \pm 33.0

Note: In the pseudopregnancy determinations, the animals being young show, in general, lower levels of eosinophil fluctuation in their normal cycles than those in the averages for all ages given above.

variations in luteinizing hormone liberated from the anterior lobe of the pituitary gland under those conditions: i.e., low in immature animals, with a sudden rise at maturity; a rise during oestrus, contributing during this rise to the process of ovulation as suggested by Hisaw, Fevold, Foster and Hellbaum (1934); a rise during pseudopregnancy resulting in the formation of functional corpora lutea. Regarding constant oestrus, divergent opinions exist. Parabiosis experiments lead DuShane, Levine, Pfeiffer and Witschi (1935) to believe that the condition is due to lack of luteinizing hormone. On the other hand, Smith and Engle (1934) find ovarian cysts resulting from implantations of anterior lobe containing probably both follicular stimulating and luteinizing hormones, while injections of castrate

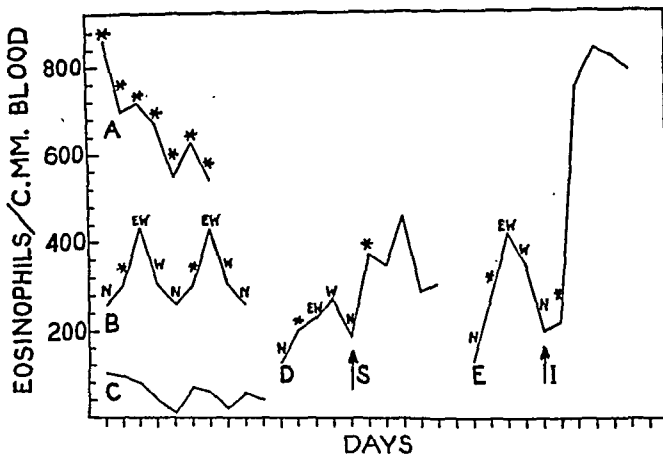


Fig. 1. Graph of figures in table 1, showing daily variations in concentration of eosinophil leucocytes under differing conditions. *A*, rats in constant oestrus. *B*, rats experiencing normal cycles. *C*, immature rats. *D*, pseudopregnancy induced by sterile copulation at (arrow) *S*. *E*, pseudopregnancy induced by serum injection at (arrow) *I*. Stages of cycle indicated by symbols: *N*—prooestrus; *—oestrus; *EW*—post-oestrus; *W*—dioestrus.

urine containing only the follicular stimulating factor do not produce this effect. A high, relatively static balance of hormonal influence on the ovary as the causative factor in continued oestrus is indicated by the results of both groups. In the present experiments, the production of pseudopregnancy and cystic ovaries by a common treatment and the high eosinophil number in constant oestrus subscribe to this concept.

The observations above led to attempts to assay the eosinophil leucocytes of the female rat qualitatively for luteinizing hormone. Isolation of these cells has never been accomplished, but white cell suspensions of relatively high and low eosinophil content were compared in respect to their power to augment the action of follicular stimulating hormone in immature female rats. Suspensions of high eosinophil concentration were obtained from the

citrated blood of pregnant, serum-injected or constant-oestrous rats by repeated centrifuging and washing three times with physiological saline solution. Low concentrations were similarly obtained from non-pregnant and saline-injected adults. Greater eosinophil concentration was sometimes obtained by the use of hypotonic (0.75 per cent) saline in washing, while hypertonic (0.9 per cent) solution tended to have an opposite effect.

The follicular stimulating hormone used was a 60 per cent alcohol-soluble fraction of an aqueous pyridine extract of acetone-dried pig anterior lobe, prepared after the method of Fevold et al. (1933).

Subcutaneous injections of follicular stimulating hormone-plus-white cells were made twice daily into 22-day old rats for 3 days, the animals being autopsied on the fifth day. Ovaries were dissected free and weighed. There was considerable variability in the response of different litters to the control doses of like amounts of hormone, but, within each litter, the results were fairly constant. ZnSO_4 , augmentative in action (Maxwell,

TABLE 2

HORMONE PLUS	ANTI-MALS	A	B	C	PER CENT AUGMENTATION	
					Range	Average
1. Over 1 million E.	11	50.85	0.177	$2.154 \pm .223$	47-246	148.2 ± 16.1
2. Under 1 million E.	9	33.2	0.167	$0.513 \pm .053$	0-63	39.6 ± 5.46
Difference				$1.641 \pm .23$		108.6 ± 17.0

A. Average of millions of white cells received by each animal.

B. Average volume of blood elements (packed by centrifuging) received by each animal.

C. Average of millions of eosinophil cells received by each animal.

1934), injected in doses of 10 mg. in combination with follicular stimulating hormone into 4 rats from 3 different litters, although resulting in ovaries which ranged from 66 to 118 mgm. in weight, showed fairly uniform augmentation percentages of 433, 464, 500 and 503 over littermate control ovaries. Therefore the per cent of increase in ovarian weight over littermate hormone-injected controls rather than gross ovarian weight has been used in table 2. The 12 controls used had a range of 12.4 to 22.6 mgm. in ovarian weight with an average of 18.02.

The response is apparently proportional to the number of eosinophil leucocytes injected. Group 1, receiving on the average 319 per cent more eosinophils than group 2, demonstrated a 274 per cent greater ovarian response, while receiving 53 per cent more white blood cells and 6 per cent more total blood cellular material. The small difference in number of white cells injected can hardly be responsible for the increased augmentation in group 1, since ovarian response does not increase proportionally as

the amount of a non-hormonal substance administered is increased, e.g., casein (Saunders and Cole, 1936). Evans, Simpson and Austin (1933) achieved augmentation with 3 cc. doses of rat blood serum, greater from non-pregnant than pregnant females. Eosinophilia, provoked by blood-group antagonisms, is indicated by these results, especially since pregnant blood is believed to be higher in luteinizing hormone content than non-pregnant. Blood-group reactions, too, are probably responsible for the wide ranges shown in table 2, but this effect should be common to both groups, 1 and 2. Therefore, the difference in response is probably due to a difference in content of luteinizing hormone.

A number of non-specific substances have displayed augmenting properties when administered to rats in conjunction with purified or unfractionated pituitary extracts. Extracts of male urine, milk, egg white, lemon, horse thyroid and beef liver (Hellbaum, 1936); various blood sera and urines (Evans et al., 1933); formed elements of beef blood (Casida, 1936); casein and egg albumen (Saunders and Cole, 1936); serum from non-pregnant mares (Cole and Hart, 1934); tannic acid (Fevold et al., 1933); and ZnSO_4 (Maxwell, 1934) have proven effective in evoking this phenomenon. The tentative explanation, a delayed absorption of follicular stimulating hormone, may account for a portion of the augmentation, but, in view of the huge increases observed in non-hypophysectomized animals in response to non-hormonal substances, these have probably initiated events resulting in a steady liberation of luteinizing hormone from the living pituitary.

As shown by Lane and Hisaw (1934), the pituitaries of immature rats can be provoked to liberate luteinizing hormone by uterine cervical stimulation and by administration of oestrin. It seems believable that the unifying mechanism of the action of various injected substances may be a stimulation to eosinophilia, these cells providing an increased amount of luteinizing hormone in the circulation. An eosinophilia, persisting for some time after treatment, is expected in the case of foreign protein injections (guinea pigs, Biggart, 1932). Cole, Guilbert and Goss (1932) found gonadotropic mare serum with a high protein content as effective in a single dose as in divided doses, suggesting again the initiation of some mechanism such as that proposed, rather than direct hormonal action. The failure of ZnSO_4 and casein in combination to act additively in augmentation (Saunders and Cole, 1936) points to the identity of method of their action. The common pharmacological action of ZnSO_4 and tannic acid in the precipitation of proteins hints at the same mechanism. Preliminary experiments in this laboratory indicate a rise in eosinophils after injections of ZnSO_4 either alone or in combination with follicular stimulating hormone into immature females, while injections of hormone alone tend to lower the eosinophil number.

In view of the evidence presented, it seems credible that the concentration of eosinophil leucocytes in the circulation of the female rat may be an indication of luteinizing activity; and further, that the cells themselves may find a rôle in the control and transport of the luteinizing hormone.

SUMMARY

1. Goat anti-rat thymus serum either by single or repeated intraperitoneal injections induced pseudopregnancy in adult female rats, with accompanying eosinophilia.

2. The concentration of eosinophil leucocytes in the circulation of female rats of various ages appeared to vary as the probable concentration of luteinizing hormone of the pituitary.

3. Assays of leucocyte suspensions for luteinizing hormone showed those of high eosinophil cell content to be more effective in augmenting the action of follicular stimulating hormone in immature females than those of low eosinophil content.

REFERENCES

- BIGGART, J. H. *J. Path. and Bact.* **35**:799, 1932.
CASIDA, L. E. *Proc. Soc. Exper. Biol. and Med.* **33**:570, 1936.
COLE, H. H., H. R. GUILBERT AND H. GOSS. *This Journal* **102**: 227, 1932.
COLE, H. H. AND G. H. HART. *Proc. Soc. Exper. Biol. and Med.* **32**:370, 1934.
DUSHANE, G. P., W. T. LEVINE, C. A. PFEIFFER AND E. WITSCHI. *Proc. Soc. Exper. Biol. and Med.* **33**:339, 1935.
EVANS, H. M., M. E. SIMPSON AND P. R. AUSTIN. *J. Exper. Med.* **58**:561, 1933.
FEVOLD, H. L., F. L. HISAW, A. A. HELLBAUM AND R. HERTZ. *This Journal*, **104**: 710, 1933.
GOLDMAN, D. O. AND C. P. KRAATZ. Unpublished work.
HELLBAUM, A. A. *Proc. Soc. Exper. Biol. and Med.* **33**:568, 1936.
HISAW, F. L., H. L. FEVOLD, M. A. FOSTER AND A. A. HELLBAUM. Supplement to no. 4, Vol. **60**, *Anat. Rec. abstracts*, 1934.
LANE, C. E. AND F. L. HISAW. *Ibid.*, 1934.
MAXWELL, L. C. *This Journal* **110**:458, 1934.
SAUNDERS, F. J. AND H. H. COLE. *Proc. Soc. Exper. Biol. and Med.* **33**:505, 1936.
SMITH, P. E. AND E. T. ENGLE. *J. Pediat.* **5**:163, 1934.

A STUDY OF THE SPEED OF ABSORPTION FOLLOWING THE INGESTION OF GLUCOSE AND OF SUCROSE

ALICE C. ROBERTS¹

From the Department of Physiology, School of Medicine, University of Chicago

Received for publication June 17, 1936

Several different methods have been utilized for the study of absorption of carbohydrates from the gastrointestinal tract. One of these is well represented by the work of Cori (1925) which utilizes the intact gastrointestinal tract. In this method, the test solution is introduced into the stomach of the fasting animal by stomach tube, is allowed to remain for varying periods of time, after which the animal is killed, and the contents of the gastrointestinal tract are analyzed.

Ravdin et al. (1933) studied absorption from a "Thiry loop," or segment of intestine, as modified by one of them (Johnston, 1932). Into a loop segment made in this manner, the fluid to be tested is introduced, is allowed to remain for a predetermined period, after which it is withdrawn for analysis.

It has long been obvious to workers in the field that, after carbohydrate ingestion, the rise in the blood sugar level and its subsequent fall must be dependent on the rate of absorption of the sugar from the intestine, on the one hand, and on the rate of loss of sugar from the blood by various physiological means, on the other. In this study we have utilized the difference in the speed of rise of the blood sugar curves from their basal levels, after ingestion of glucose and of sucrose, as being indicative of the relative rates of absorption of those two sugars.

EXPERIMENTAL. The experimental animals were picked at random and therefore varied greatly in breed, age, and size. However, all four of the dogs were males. Their diet consisted of bread, lung, and bone meal, supplemented with cod liver oil. They received approximately fifteen minutes' exercise a day. The animals were trained to lie quietly over a period of several hours. Before each experiment they received no food for about twenty hours.

After being brought from the cage for the experiment, the dogs always rested for a half-hour period before the basal sample of blood was procured. As soon as this sample (1) had been drawn, the test fluid (6 grams per kilo of the sugar to be tested, in about 60 per cent aqueous solution) was intro-

¹ Fellow, Corn Products Research Fund.

duced into the animal by stomach tube, whereupon the dog was returned to its resting position in which it remained until the end of the experiment. At exactly one-half hour and one hour after the animal received its sugar, samples 2 and 3 respectively were taken for analysis. The blood was analyzed by the Shaffer-Hartmann method as modified by Somogyi (1921-1933).

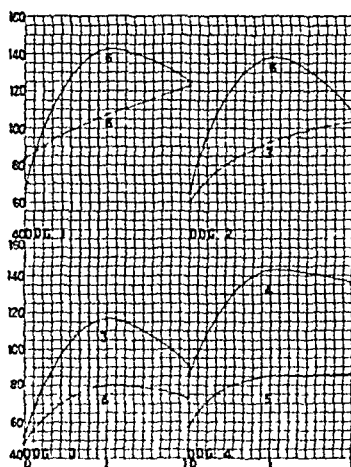


Fig. 1. Dog's blood sugar after ingestion of 6 grams per kilo of corn sugar and cane sugar. The curves are averages of the number of experiments indicated by the figures placed on each curve.

RESULTS. The composite blood sugar curve of each animal, based upon blood sugar determinations following numerous glucose ingestions, rises more sharply and to a greater height during the first half-hour after glucose administration, than the comparable curve rises following sucrose ingestion.

DISCUSSION. In contrast to the work of Ravdin et al. (1933), the present investigation was undertaken to study the relative speed of absorption of glucose² and sucrose in the normal, unanesthetized dog with its entire gastrointestinal tract functioning as a unit. Since it was desired to compare the action of two different sugars in the same dog, an acute experiment such as Cori performed would have been impractical. Also Cori's statistical treatment, made possible by the use of small animals (rats), would not have been feasible where dogs were the subjects. The method which was adopted for this study, the use of the rate of rise of the blood sugar curve as the indicator of the speed of absorption, allowed a large number of experiments to be performed on the same animal and utilized the normal gastrointestinal tract of the unanesthetized dog.

The choice of the amount of sugar, though quite arbitrary, was made to insure an excess of the sugar during the absorption period. The high concentration was deemed advisable, since the dogs which had a tendency

² "Dyno," a product of the Corn Products Company, was the source of glucose.

to regurgitate seemed better able to retain their sugar when the volume of the solution was limited. Cori (1925) had found that the absorption was not influenced by the absolute amount or by the concentration of the sugar present in the intestine.

When the stomach tube was administered to the animal, but no sugar was given, the rise in blood sugar was found to be negligible.

Although we agree with the position of Trimble et al. (1933), as based on their work and that of Johansson (1909), that there is probably "a lack of significance in its (the blood sugar curve's) return to normal as an indicator of the completion of the absorption process," our own results are not concerned with those portions of the sucrose and glucose curves in which there is a return to the basal blood sugar level, but rather with the rising portions of these curves, and their relative heights at the end of the first half-hour after administration of the test solution. During this early period that still elusive mechanism, which tends to throw the blood sugar curve back to normal before absorption has stopped or even slowed, can scarcely be responsible for the fact that the blood sugar curve rises more slowly after sucrose than after glucose ingestion, since the influence of this mechanism to throw the curve back to normal is much more marked in the case of glucose than it is in the case of sucrose. It would seem from the curve, then, that this mechanism has not become an important factor during the period to which we are confining our attention and from which we are drawing our conclusions.

It has been shown that a large percentage of carbohydrate entering the blood stream (by injection) disappears rapidly therefrom (Bang, 1913). However, Cori (1927-28) has demonstrated that this may be recovered from the tissues and that all sugars penetrate into the tissues at the same rate, i.e., the tissues are not selective in taking up different sugars as are the intestines. Therefore, while this fact would affect the use of the blood sugar level as an indicator of the absolute rate of absorption, it does not prevent the use of the blood sugar level as an indicator of which sugar is entering the blood stream more rapidly through the intestines.

SUMMARY

Numerous experiments have been carried out to determine the relative blood sugar level after glucose and after sucrose ingestion in the unoperated, unanesthetized dog. There appears to be a very definite tendency toward a higher blood sugar level after glucose than after sucrose ingestion. This difference is interpreted to be due, at least in part, to the faster rate of absorption of glucose than of sucrose from the gastrointestinal tract.

I wish to express my appreciation to Dr. A. J. Carlson for suggesting this problem and for his valuable advice throughout the course of the work

REFERENCES

- BANG, I. *Der Blut Zucker*. Wiesbaden, 1913.
- CORI, C. F. *J. Biol. Chem.* **66**:691, 1925.
Harvey Lectures, 1927-28, 78.
- JOHANSSON, J. E. *Skand. Arch. Physiol.* **21**: 1, 1909.
- JOHNSTON, C. G. *Proc. Soc. Exper. Biol. and Med.* **30**: 193, 1932.
- RAYDIN, I. S., C. G. JOHNSTON AND P. J. MORRISON. *This Journal* **104**: 700, 1933.
- SHAFFER, P. A. AND A. F. HARTMANN. *J. Biol. Chem.* **45**: 349, 365, 1921.
- SHAFFER, P. A. AND M. SOMOGYI. *J. Biol. Chem.* **100**: 695, 1933.
- SOMOGYI, M. *J. Biol. Chem.*, **52**: 599 1926; *Proc. Soc. Exper. Biol. and Med.* **26**: 353, 1929; *J. Biol. Chem.* **75**: 33, 1927; **78**: 117, 1928; **80**: 733, 1928; **83**: 157, 1929; **86**: 655, 1930; **90**: 731, 1931; **70**: 599, 1926.
- TRIMBLE, H. C., B. W. CAREY AND S. J. MADDOCK. *J. Biol. Chem.* **100**: 124, 1933.

ELECTRICAL STIMULATION OF THE INTERIOR OF THE CEREBELLUM IN THE DECEREBRATE CAT¹

W. K. HARE, H. W. MAGOUN AND S. W. RANSON

From the Institute of Neurology, Northwestern University Medical School

Received for publication June 19, 1936

Responses to faradic stimulation of the interior of the cerebellum of the monkey have been described by Magoun, Hare and Ranson (1935). The present paper is concerned with the reactions similarly obtained in normal and decerebrate cats.

Stimulation within the cerebellar nuclei and the adjacent fiber paths was accomplished with the aid of the Horsley-Clarke stereotaxic apparatus, the use of which has been described by Ranson (1934). As in the experiments on the monkeys, a needle-like bipolar electrode, the exposed tips of which were 1 mm. apart, was used to conduct the exciting current led off from the secondary coil of a Harvard inductorium. The primary coil, connected to a single dry cell providing a flow of 1.5 ampere when in circuit with the inductorium, was 9 cm. from the secondary. Stimuli were applied at millimeter intervals along the course of each puncture so that a thorough exploration was obtained in each case. The cerebellum was prepared for microscopic examination and the position of the tips of the electrodes during each stimulation was anatomically identified. Of the twelve decerebrate cats used, ten were stimulated with vertical electrodes; the other two decerebrate and all the normal cats were stimulated with horizontal electrodes. All of the cats were suspended by the frame of the stereotaxic instrument and by strings passing under the supraspinous ligaments, an arrangement which permitted free movements of all parts of the body.

The decerebrate cats were operated under ether anesthesia which was discontinued when the brain stem was severed so that during the period of stimulation the animal, though completely unconscious, was not subjected to the depressing effect of an anesthetic.

All the reactions to cerebellar stimulation present two definite phases: a first which is coincidental with the electrical stimulation, and a second, usually the contrary of the first, which appears as a rebound after the cessation of the stimulation and which continues for a time varying from a few seconds to five minutes or more. It became apparent during the course of the experiments that the majority of the cerebellar responses were readily

¹ Aided by a grant from the Rockefeller Foundation.

divisible into two great groups; one consisting of the responses of the head, trunk, tail and all four extremities in a manner closely similar to the tegmental reaction (Ingram, Ranson, Hannett, Zeiss and Terwilliger, 1932), and another including the reactions of the ipsilateral forelimb not admissible to the first. That such separation of the responses is at least partly natural and not wholly artificial is evidenced by the localization of the first group near the midline in the vermal portion of the cerebellum and of the second group in the lateral parts of the vermis and the most medial parts of the interior of the cerebellar hemispheres.

Within the first category a very great number of the responses are in conformity with the following pattern: During stimulation there is a flexion of the ipsilateral limbs, extension of the contralateral, deviation of the head, trunk and tail with the concavity toward the side of stimulation. The rebound, lasting for several seconds to several minutes, makes its appearance only after the end of the electrical stimulation and consists of extension of the ipsilateral limbs, flexion of the contralateral and formation by the vertebral axis of an arc concave to the side opposite the point stimulated. A response fulfilling this pattern in every detail is illustrated in photographs G and H of figure 1. The pictures, all of decerebrated cats, are arranged in pairs representing the two phases of the reaction to stimulation of a single point within the cerebellum. The upper photograph of each pair was taken during the first phase, the lower during the rebound. The point stimulated to produce the postures shown in G and H was in the left side of the cerebellum. In photograph G, taken while the cerebellum was being stimulated, the head, and concavity of the trunk and tail are directed to the left; the left or ipsilateral limbs are powerfully flexed, and the right legs are extended. In the rebound, illustrated in photograph H, the animal assumed a posture the opposite of that obtaining during stimulation; the entire body axis is an arc concave to the right, the right limbs are flexed, and the left are in full extension.

A less vigorous response of the same pattern is illustrated in E and F of figure 1, but in this instance the electrodes were in the right side of the cerebellum. A still weaker reaction to faradization within the right side of the cerebellum is seen in C and D. Stimulation caused generalized inhibition or relaxation but the rebound posture was that of tonic extension of the right limbs, flexion of the left, and a slight concavity of the spinal column to the left. Stimulation of some points in the cerebellum produced such weak or attenuated reactions that only one member of the body would participate. Turning of the head to the right during stimulation when the electrodes were in the right side of the cerebellum is portrayed in photograph A; the rebound, in which the head is turned to the left, is shown in B.

On following the photographs in the sequence in which they have been described it is obvious that the reactions become progressively weaker and

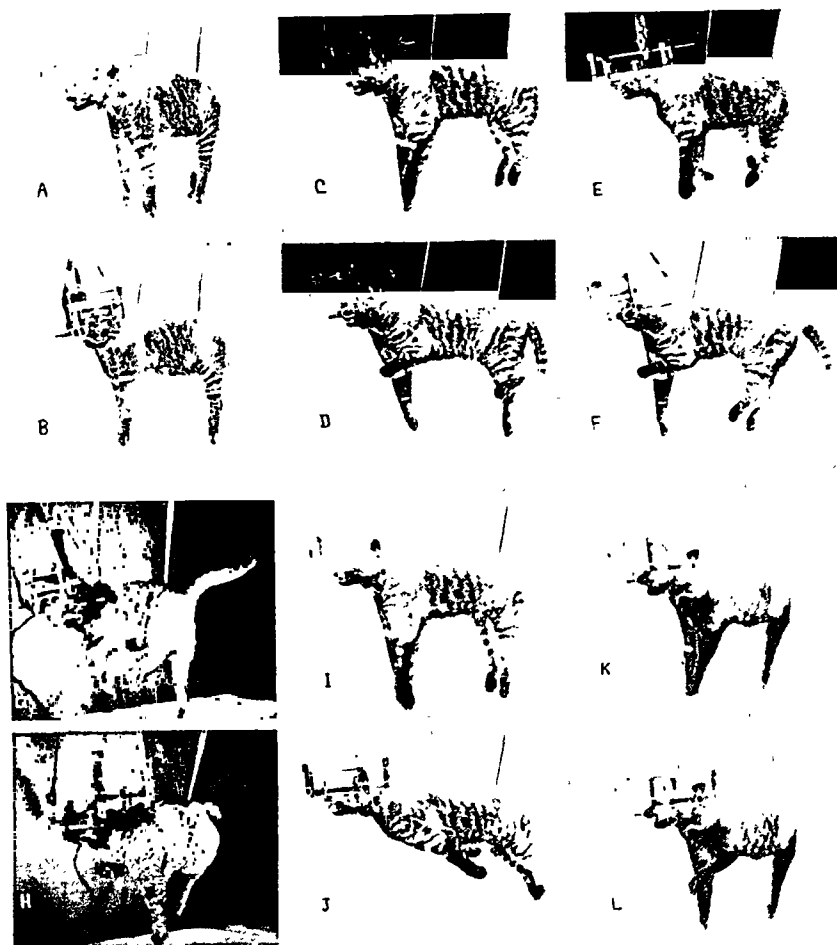


Fig. 1 contains six pairs of photographs portraying the responses of the decerebrate cat to cerebellar stimulation. Each pair represents the two phases of the response to stimulation of a single point within the interior of the cerebellum, the upper picture in each case showing the first phase which is coincidental with stimulation, and the lower one the second phase or rebound occurring after stimulation. All of the photographs except K and L show reactions obtained from the cerebellar vermis. Pictures A and B show a weak response, in which only the head is active, to a stimulus applied in the right side of the cerebellum. C and D also illustrate a response to stimulation in the right side of the cerebellum but in this case the limbs took part in the reaction. Photographs E and F present a still stronger reaction to a stimulus in the right side of the cerebellum. During the first phase the right limbs are slightly flexed, the left extended, and the vertebral column curved to the right. On the rebound the opposite prevails; the right limbs are extended, the left are flexed and the concavity of the trunk is directed to the left. A response of maximal strength is shown in G and H, illustrating a response to stimulation in the left side of the cerebellum. Photographs I and J show the type of reaction most often seen on stimulating near the sagittal plane and which consists of relaxation during stimulation followed by a rebound in which the two halves of the body respond in a similar manner, producing a symmetrical posture. The last pair of pictures, K and L, represents an ipsilateral forelimb response obtained from the medial part of the left cerebellar hemisphere.

involve fewer parts of the body but it is to be stressed that they are all of the same basic pattern and differ only in intensity.

While the majority of the reactions to cerebellar stimulation are composed of postures effected by the flexors and extensors of the limbs, it should be noted that pronation, supination, adduction and abduction of the limbs, torsion of the spinal column and rotation of the tail are not uncommon and that they appeared in the rebound as well as during the period of stimulation.

The level of decerebration, microscopically determined, was in no way related to the production of this type of response to cerebellar stimulation for the pattern, intensity and duration of the reactions were the same whether the brain stem was severed through the thalamus, at the rostral end of the midbrain or at the anterior margin of the pons. It seems certain that exclusion of the red nuclei does not alter cerebellar responses of this type for after transection of the brain stem caudal to the red nuclei responses of this kind were found to be just like those seen in decerebrate cats in which the red nuclei were intact.

Apart from the responses just described, there are others which can perhaps best be regarded as aberrant forms as, for example, those elicited when the stimulation was almost or exactly in the median sagittal plane. Often on such occasions the postures did not show the asymmetry of the typical reaction, neither in the first nor in the second phase. In other cases they were asymmetrical in one phase and symmetrical in the other. Such a reaction in which the right and left halves of the body responded in an identical fashion is shown in I and J.

When stimuli were applied in the lateral parts of the vermis or within the medial parts of the cerebellar hemispheres, postures of a strikingly different character were often produced. Throughout the duration of the stimulus the ipsilateral foreleg was either relaxed or slightly extended; on the rebound the limb was tonically flexed and sometimes adducted. This flexor rebound contrasted sharply with the strong extension of the limb obtained at the end of a stimulus placed a little more medially in the same side of the cerebellum. Other parts of the body took no part in either phase of this type of reaction which is illustrated in K and L. The tips of the stimulating electrodes in this case were in the left side of the cerebellum and it is only the left foreleg which is reactive. The frequent occurrence of these responses led to their recognition as the expression of a mechanism quite distinct from that activated by stimuli in the more medial parts of the cerebellum. The complete disappearance of this type of response as the electrodes approached the cerebellar peduncles makes it impossible to identify the efferent cerebellar tracts over which it is mediated but this ipsilateral forelimb reaction was not observed in any of the decerebrate cats in which the level of transection was caudal to the red nuclei. It was repeatedly obtained in one cat in which only the caudal poles of the

red nuclei were present but because of the possibility that these cells were not functional as the result of hemorrhage and trauma the evidence is not clear as to the participation of the red nuclei in this reaction. It must be taken into consideration that the path may be by way of the brachium conjunctivum and the fibers which branch from it below the level of the red nuclei and descend in the brain stem.

Some variations from the typical reactions to cerebellar stimulation which make their appearance after considerable damage has been done to the cerebellum by repeated punctures, have been described by Magoun, Hare and Ranson (in press) and need not be discussed here.

The cerebellar exploration which had been performed on decerebrate cats was extended to a series of cats with intact nervous systems. If one leaves out of account those decerebrate cats in which all of the cells of the red nuclei were removed and in which the isolated ipsilateral forelimb responses were missing, the reactions of the normal and decerebrate cats were so similar that only careful examination permitted the observer to differentiate between them. The minor differences which were consistently seen can be explained as the consequences of slight modifications of experimental conditions. The normal cats were anesthetized with nembutal which depressed the spontaneous and reflex activity of the animals throughout the experiment. Probably because of this, the strength of the responses was considerably decreased and full development of the postures of both phases was slower and more deliberate than in the decerebrate cats. Also the rebounds were of slightly shorter duration. Since all the cranial nerves were functional in the normal cats, cerebellar excitation caused ocular responses in addition to the reactions of the trunk and limbs which have been described. Usually there was a deviation of the eyes toward the side stimulated during the first phase and a rebound toward the opposite side at the end of the stimulus.

When the tips of the electrodes were in the fourth ventricle or in the medulla oblongata itself, stimulation produced twitchings of the muscles supplied by the cranial nerves. These responses were entirely different in character from those evoked by stimuli within the cerebellum.

DISCUSSION. 1. Responses to cerebellar stimulation in lightly anesthetized cats with intact nervous systems have been found to be essentially similar to those obtained in a like manner in the monkey.

2. Identical reactions from lightly anesthetized cats with intact nervous systems and decerebrate cats show that the tonic background of decerebrate rigidity is not essential for the demonstration of cerebellar reactivity and support the suggestion made in regard to responses obtained from the monkey that these reactions to cerebellar stimulation are the same ones which have been inadequately described as the cerebellar inhibition of decerebrate rigidity.

3. Unimpaired activity of the medially situated cerebellar system after

transection of the brain stem behind the red nuclei demonstrates that a cerebello-rubral pathway is unnecessary for the mediation of its influence as was suggested by Cobb, Bailey and Holtz (1917) and indicates that its activity is discharged largely if not entirely by cerebello-bulbar connections, confirming Bernis and Spiegel (1925).

CONCLUSIONS

From the evidence derived from electrical stimulation of the cerebellum of the cat there seem to exist in that organ two neural mechanisms capable, when properly excited, of producing and maintaining biphasic postural responses of two very distinct patterns. The first of these is related to the entire axial and appendicular musculature; the influence of the second is restricted to the ipsilateral foreleg. Decerebration, even when the brain stem is severed caudal to the red nuclei, does not affect the activity of the first other than perhaps to add to its intensity, nor does decerebration appear to alter the expression of the second unless the transection entirely excludes the rubral apparatus, in which case the rebound flexion of the ipsilateral foreleg is no longer obtainable.

REFERENCES

- BERNIS, W. J. AND E. A. SPIEGEL. *Arch. a. d. Neurol. Inst. a. d. Wien. Univ.* 27: 197, 1925.
- COBB, S., A. A. BAILEY AND P. R. HOLTZ. *This Journal* 44: 239, 1917.
- INGRAM, W. R., S. W. RANSON, F. I. HANNETT, F. R. ZEISS AND E. H. TERWILLIGER. *Arch. Neurol. and Psychiat.* 28: 513, 1932.
- MAGOUN, H. W., W. K. HARE AND S. W. RANSON. *This Journal* 112: 329, 1935.
- Arch. Neurol. and Psychiat. *In press.*
- RANSON, S. W. *Psychiat. en neurol. bl.* 38: 534, 1934.

THE SPINAL PATH FOR RESPONSES TO CEREBELLAR STIMULATION¹

E. H. INGERSOLL, H. W. MAGOUN AND S. W. RANSON

From the Institute of Neurology, Northwestern University Medical School

Received for publication June 29, 1936

A recent series of studies has indicated the important influence which an electrically excitable, medially situated cerebellar system is capable of exerting upon efferent centers for the contraction of axial and appendicular musculature. Investigation of the activity of this system in the decerebrate animal (Hare, Magoun and Ranson, 1936) has shown it to be unimpaired after removal of the red nucleus, which suggests that its influence may be mediated, in large part at least, by cerebello-bulbar and bulbo-spinal connections. In an effort to gain further information concerning the spinal pathways involved, the effect of lesions of the first cervical segment of the spinal cord upon the responses to cerebellar stimulation has been investigated in the cat.

METHOD. Using the Horsley-Clarke stereotaxic instrument (Ranson, 1934), responses to faradic stimulation of either side of the interior of the medial part of the cerebellum were observed following section of various parts of the spinal cord at the first cervical level in each of fifteen cats under light nembutal anesthesia. All of the experiments were performed acutely, the reactions being tested from fifteen to forty-five minutes following cord section. Control stimulation of the same foci in the cerebellum after exposure of the cord, but before section, had revealed characteristic responses. Suspension of the animals in the air by support of the head through the stereotaxic instrument and of the body by cords passed through the supraspinous ligament at the shoulder and pelvis, permitted complete freedom of movement of the limbs.

All spinal cord lesions were made on the left side. For hemisection and section of the lateral funiculus, the dorsal neck muscles were reflected and the caudal portion of the dorsal arch of the atlas was removed, permitting wide transverse section of the dura. For section of the ventral funiculus, a tracheal cannula was inserted, the larynx and esophagus were retracted to the right, the carotid sheath to the left and the vertebral muscles were reflected from the atlas and second vertebra. The ventral arch of the atlas and the odontoid process of the second vertebra were removed, and

¹ Aided by a grant from the Rockefeller Foundation.

the dura was sectioned in the midline from the body of the second vertebra to about the position of the rostral edge of the atlas. Retraction of the dura with fine forceps then gave an excellent exposure of the ventral aspect of the spinal cord. Cord sections were made with the aid of two fine scalpels, one straight with and the other at a right angle to the handle. The extent of each lesion was determined microscopically by a study of serial Weil-stained sections.

OBSERVATIONS. The response to stimulation of one side of the medial portion of the interior of the cerebellum is a biphasic one, one phase occurring during the period of stimulation, and the second phase being manifest as a rebound at the cessation of stimulation. This rebound consists, at its maximal strength, of a pronounced and longlasting extension of the ipsilateral limbs, a flexion of the contralateral limbs, and a contraction of the contralateral axial muscles. All of these postures are relaxed during stimulation and at the same time a contraction of the antagonists of these muscles often occurs. Variations in the responses consist chiefly in the extent to which inhibition or excitation is manifest, and in the extent to which all parts of the body participate. The effect of cord lesions on these responses may now be described.

Hemisection. Following hemisection of the first cervical segment of the spinal cord in each of four animals the reactivity of the limbs on the side of section was consistently abolished to stimulation of either the ipsi- or contralateral half of the cerebellum. The reactivity of the limbs opposite the side of section was consistently retained to stimulation of either half of the cerebellum. While possible "shock" effects associated with acute hemisection should not be overlooked, the results indicate that pathways which mediate the electrically excitable influences from either half of the cerebellum to the limbs on one side of the body are contained exclusively in the half of the spinal cord on that side.

With the establishment of a syndrome of hemisection, the attempt was made to delimit the significant portion of the hemisected area responsible for the deficit.

Section of the lateral funiculus. Following complete section of the lateral funiculus in each of two animals no impairment could be observed in the responses on either side of the body to activation of either half of the cerebellum.

Section of the ventral funiculus. Following section of the ventral funiculus which was complete to the midline in one animal and almost so in three others, the reactivity of the limbs opposite the side of section was not affected. The reactions of the forelimb on the side of section to stimulation of the ipsilateral half of the cerebellum were retained in all four of the animals, in two occasionally approaching the vigor of response seen before section, and in the other two being of consistently reduced intensity.

The reactions of the hindlimb on the side of section to stimulation of the ipsilateral half of the cerebellum were abolished in two animals, but were retained in the other two. The reactivity of the fore and hind limbs on the side of section to stimulation of the contralateral half of the cerebellum was considerably affected. Responses of the forelimb were consistently reduced in intensity, and lost all participation of flexor contraction in the rebound. Reactions were abolished in the hindlimb in two animals.

While section of the ventral funiculus has exhibited some variability of effect, it is apparent that this lesion causes an impairment not seen after lesions confined to the lateral funiculus of the spinal cord. It is obvious, however, that the effect of section of either the ventral or the lateral funiculus alone is not equivalent to that of hemisection of the cord.

Combined lesions of ventral and lateral funiculi. In three animals sections were made from the dorsal approach, interrupting not only the lateral funiculus but extending ventrally to involve about the lateral third of the ventral funiculus as well. The reactivity of the limbs opposite the side of section was not affected. In one of the animals the reactivity of the forelimb on the side of section was abolished to stimulation of either side of the cerebellum. In the other two animals with lesions almost identical to the first, the reactivity of the fore and hind limbs on the side of section was retained to stimulation of the ipsilateral side of the cerebellum, although usually reduced in intensity, and was abolished to contralateral stimulation.

In two animals sections were made from the ventral approach interrupting not only the ventral funiculus but extending dorsally to involve about the ventral half of the lateral funiculus as well. The reactivity of the limbs opposite the side of section was not affected. The forelimb on the side of section responded to stimulation of the ipsilateral half of the cerebellum only by a very slight rebound forward movement in the first animal and by a slow rebound extension and stepping in the second. To stimulation of the opposite half of the cerebellum this forelimb responded with a phasic rebound abduction in the first animal, and by a slight gain in extensor tone during stimulation in the second. In both animals, therefore, responses of the forelimb on the side of section were severely impaired. All reactivity of the hind limbs on the side of section was abolished.

Combined lesions of the lateral and ventral funiculi are seen, therefore, to produce a degree of impairment greater than that occasioned by sections confined to either funiculus alone, and lead to a deficiency in the reactivity of the limbs on the side of section which approaches that induced by hemisection of the cord.

The results clearly indicate that the pathways which transmit the effects of cerebellar stimulation to spinal levels are diffusely represented in both the lateral and ventral funiculi of the upper cervical cord, possibly with a

greater concentration in the ventral funiculus. It is of interest to note the similarity of this distribution with that of the reticulo-spinal tracts (Papez, 1926), more especially since the reticular formation of the brain stem has been emphasized as an important site of distribution of efferent fibers from the cerebellum (Allen, 1924, 1932; Rasmussen, 1933). Possible participation of descending connections from the vestibular nuclei should not be overlooked, however. The topographical data provided by the present experiments does not permit one to draw any final conclusions concerning the specific fiber tracts involved.

Interruption of ascending proprioceptive pathways does not appear to have been a factor of any importance in these experiments. Interruption of the dorsal spino-cerebellar tract in sections of the lateral funiculus has left the responses of the limbs on this side, as well as on the opposite side, unimpaired. Interruption of the ventral spino-cerebellar tract in many of the sections did not influence the reactions of the opposite limbs. It has been shown, moreover, in another investigation (Magoun, Hare and Ranson, 1936) that complete deafferentation of a limb does not affect its response to cerebellar stimulation other than to reduce the duration of rebound contraction.

SUMMARY

Following hemisection of the first cervical segment of the spinal cord, the responses of the limbs on the side of section to stimulation of either half of the cerebellum are abolished. The reactivity of the limbs opposite the side of section is retained.

Section of the lateral or ventral funiculus alone does not produce this deficiency, while combined but incomplete lesions of these funiculi cause impairment which approaches it.

It is concluded that at the upper cervical level, the pathways which transmit the effect of stimulation of either side of the cerebellum to spinal centers are diffusely represented in both the lateral and ventral funiculi of the half of the cord on the reactive side.

REFERENCES

- ALLEN, W. F. *J. Comp. Neurol.* **36**: 399, 1924.
J. Washington Acad. Science **22**: 490, 1932.
HARE, W. K., H. W. MAGOUN AND S. W. RANSON. *This Journal.* In press.
MAGOUN, H. W., W. K. HARE AND S. W. RANSON. *Arch. Neurol. and Psychiat.*
In press.
PAPEZ, J. W. *J. Comp. Neurol.* **41**: 365, 1926.
RANSON, S. W. *Psychiat. en neurol. bl.* **38**: 534, 1934.
RASMUSSEN, A. T. *J. Comp. Neurol.* **57**: 165, 1933.

BLOOD FLOW IN THE CIRCUMFLEX BRANCH OF THE LEFT CORONARY ARTERY OF THE INTACT DOG

HIRAM E. ESSEX, J. F. HERRICK, EDWARD J. BALDES AND
FRANK C. MANN

*From the Divisions of Experimental Medicine, Physics and Biophysical Research,
The Mayo Foundation, Rochester, Minnesota*

Received for publication July 6, 1936

The thermostromuhr method of Rein has made possible an attack on the problem of coronary inflow since units can be placed on the main coronary arteries or their branches. Rein has done a series of experiments using his method to determine the blood flow in the right coronary artery of the dog. Hochrein and Keller, using Rein's method, studied the blood flow in the left coronary artery.

Previous studies on the coronary blood flow have been largely devoted to analyses of the factors responsible for variations in flow. Such investigations were of necessity confined to animals under general anesthesia. Every physiologist who has given the matter consideration realizes that data obtained from animals under general anesthesia represent at best only an approximation of what occurs in the normal, active, conscious organism. Consequently, in all our studies with the Rein stromuhr we have made every effort to determine the blood flow under as nearly normal conditions as we could devise. Our approach to the problem of coronary blood flow has been from a synthetic rather than from an analytical point of view. We are reporting here data on the blood flow in the circumflex branch of the left coronary artery of the intact dog in response to a variety of stimuli such as drugs, food, and exercise.

METHODS. In order to study the coronary blood flow it was necessary to develop a suitable unit for placing on the coronary arteries. It was likewise essential that the units be constructed so that they could be sterilized by boiling. A unit of the type used in our other experiments was not suitable for the coronary arteries. In order to have a minimal thickness of material interposed between the artery and the myocardium it was necessary to make the cuff that fits around the artery as thin as practicable. The multiplicity of lateral branches leading from the coronary arteries requires that the axis of the unit paralleling the vessel be as short as possible.

A unit was eventually designed to meet these specifications. It has been

our experience that units with internal diameters ranging from 1.0 to 2.5 mm. are of sufficiently variable size for use on the coronary arteries of dogs ranging from 6 to 20 kgm. in weight.

OPERATIVE PROCEDURES. Under ether anesthesia and with the usual aseptic technic, an adequate left lateral incision is made between the fifth and sixth ribs. The pericardium is incised over the region of the left coronary artery. The edges of the incised pericardium are then grasped and sufficient traction is exerted to bring the heart into the desired position. A serrefine is placed on the left auricular appendage, which is drawn out of the field of operation. The circumflex branch of the left coronary artery is then identified and dissected free. A short distance distally from the origin of the circumflex branch one or two lateral branches are usually given off, and the dissection is preferably made distal to these small branches. The artery is then placed in the unit and a ligature is passed under and around the middle of the unit and tied securely. The chest is then closed in the usual manner. Observations may be made if desired while the animal is still under anesthesia. We have usually made observations immediately after the operation and during the initial stages of recovery.

Effect of the unit on the general condition of the animal. As might be expected, the presence of a foreign body in the chest usually produces sufficient irritation to cause considerable pleural effusion until the leads and the unit are walled off. As a rule spontaneous drainage occurs and the animal progresses very well. It takes food and water, runs about the laboratory, and behaves in such a normal manner that a casual observer would not suspect the presence of the unit in the thorax. Some of the animals have gained weight during the course of the experiment. Typically the heart rate is considerably more rapid and irregular soon after the operation. In some cases a normal rate is resumed after a few days and the heart again becomes regular, whereas in other instances the increased rate and irregularity persist. The temperature taken by rectum may show a slight elevation above the normal range, which in the dog is from 100° to 102°F. (protocol 1).

RESULTS. *Blood flow immediately following operation.* In all of the animals studied the blood flow was greater immediately after the operation than it was following complete recovery from the ether anesthesia. The immediate postoperative values were frequently as much as three to four times those obtained twenty-four hours later. Because of the necessary trauma incident to placing the unit on the vessel, it cannot be stated that the anesthetic was solely responsible for the greater blood flow. Representative data are given in protocol 1.

Blood flow in the quietly resting dog. Following operation, daily (except Sunday) observations were usually made while the animals rested quietly on a table for an hour or more. In a number of instances we obtained daily

PROTOCOL 1
Coronary blood flow in the quietly resting animal

DOG	WEIGHT	TIME AFTER OPERATION	PULSE RATE, BEATS PER MINUTE	AVERAGE BLOOD FLOW	RECTAL TEMPERATURE	CONDITION
	<i>kgm.</i>	<i>hours</i>		<i>cc. per minute</i>	<i>°F.</i>	
5	14.5*	4		163		
		6		114		
		24	86 to 108	89	102.8	Good
		48	96 to 112	84	102.3	Eats well
		72	110 to 114	82		Eats well
		96	102 to 112	75		Eats well
		144	86 to 100	80	102.6	Eats well
		192	104 to 108	38	103.1	Eats well
		216	70 to 80	26	103.4	Eats well
		240	70 to 82	43	103.0	Eats well
		336	64 to 78	34	102.0	Eats well
10	15.4	Immediately		115		
		2		85		
		4	112	65	100.2	Good
		24	160 to 200	82	101.8	Eats well
		48	200+	63	102.0	Eats well
		120	150 to 160	76	102.1	Eats well
		144	102 to 120	91	101.6	Eats well
		168	98 to 106	80	102.0	Eats well
		192	74 to 88	85	102.0	Eats well
		216	76 to 86	80	101.0	Eats well
12	17	Immediately		90		
		24	110 to 130	66	103.5	
		48	130 to 140	75	103.3	
11	18.2	Immediately		74		Excellent
		4		61		
	18.2	24	180 to 186	50	104.2	
		48	160 to 200	53	103.7	
	19.0	72	120 to 140	64	102.6	
	20.0	96	102 to 116	76	102.2	
8	9.6	Immediately		77		
		6		28		
		24	118	23	103.5	
		48		21	103.5	Developed distemper
		72	114 to 120	30	104	
		144	96 to 102	40	103.8	
		192	142 to 200	47	105.2	Died

* This animal was killed 5.5 months after placing unit on coronary artery and necropsy was performed immediately: The unit was free from the coronary artery, which had been obliterated. The proximal end of the vessel had become occluded and was imbedded in a small mass of scar tissue. The unit was found on the edge of the pericardium imbedded in a small nodule of adhesions. There was no infection within the pleural cavity. Grossly the heart appeared normal but, on palpation, a firm area was found at the site where the unit had been applied.

values that fell within the experimental error of the method (10 per cent). In one series of observations we obtained comparable rates of flow on each succeeding day up to ten days after the unit was placed on the coronary vessel. Occasionally there occurred a reduction in the rate of blood flow by the fifth or sixth postoperative day. We have taken a gradual decrease in coronary flow to indicate that the lumen of the vessel is reduced, probably as a result of tissue reaction to the unit (protocol 1).

Complete occlusion of the coronary artery occurred in two cases within a week after operation. One of the animals was running about the laboratory when the occlusion occurred; it appeared to have been in splendid condition and had manifested intense interest in its surroundings, but suddenly it leaped, barked and succumbed. Necropsy immediately after death revealed complete occlusion of the coronary vessel to which the unit was applied.

Effect of certain drugs on coronary blood flow. Epinephrine. It has been demonstrated by numerous investigators that injections of epinephrine cause an increase in blood flow through the coronary vessels of anesthetized animals. Consequently we were interested in determining the effect of epinephrine on the coronary inflow of the intact dog. Repeated injections of 0.05 to 0.1 cc. of a 1 to 1000 solution of epinephrine caused a marked transient augmentation of from two to four times the control values. The augmentation in flow did not last longer than seven minutes in any of our experiments. As a rule the major effect on the coronary flow had terminated three minutes after injection of the drug, thus paralleling the known effect of such doses of epinephrine on the blood pressure of the dog.

Nitrites. 1. Nitroglycerine: Repeated observations under the conditions of our experiments have indicated that nitroglycerine in intravenous doses of 0.22 mgm. results in a temporary increase in coronary blood flow which at its maximum is about twice the control value. However, the increase is of short duration, since it usually is over in four or five minutes after the injection.

2. Amyl nitrite: The results of our initial experiments with this drug were open to the criticism that the nasal passages of the animals were not cocaineized before inhalation of the amyl nitrite vapor was begun. It is conceivable that reflexes resulting from the stimulation of the respiratory passages might affect the coronary flow, such as movements or failure of the animal to respire normally. In a series of later observations the nasal passages of the dogs were cocaineized by means of cotton swabs soaked in a 5 per cent solution of cocaine. Additional cocaine solution was introduced by syringe. After fifteen minutes the swabs were removed and the mask was placed over the dog's muzzle. The mask did not cause an appreciable change in coronary flow. After the mask had been in place for a short time an ampule of amyl nitrite was broken in it and inhalation followed

without alteration in the depth of respiration. Within two minutes after the amyl nitrite was introduced the coronary blood flow had increased to about twice the control values. Although the animal continued to breathe the drug, the blood flow did not remain at twice the control level for more than two minutes when it declined to a value 20 to 30 per cent above the control. On removal of the mask the blood flow approximated the control values within five minutes. In one experiment a second ampule was broken in the mask six minutes after the effect of the first ampule had been dissipated. Only a slight elevation in coronary blood flow followed. From a control of 70 cc. the blood flow increased to only 86 cc. per minute.

PROTOCOL 2

Effect of inhalation of amyl nitrite on coronary blood flow

(Dog number 12, weight 17 kgm.)

TIME	BLOOD FLOW		REMARKS
	Control	During inhalation	
	<i>cc. per minute</i>	<i>cc. per minute</i>	
11:25	60		
11:40	63		Nasal passages cocaineized for 15 minutes
12:10	66		
12:15	71		Mask on
12:18	71		Ampule of amyl nitrite broken in mask
12:18.5		103	
12:19.5		137	
12:21.5		88	
12:23			Mask off
12:25		68	
12:31		70	Mask on; second ampule amyl nitrite broken in mask
12:32		86	
12:33		80	
12:34		86	
12:36		70	Mask off

These experiments clearly indicate that amyl nitrite is capable of producing a temporary increase in coronary blood flow in the intact dog, but it appears that the dog is refractory to a second dose when it is given too soon after a previous one (protocol 2).

Thyroxin. In a previous paper (3) we have shown that the blood flow in the femoral artery of a series of six dogs was increased from 205 to 327 per cent over the control figures.

Because of the time required to produce hyperthyroidism, an adequate study of the effect of thyroxin on coronary blood flow has not been possible by the use of previous methods. In a series of experiments we have

studied the changes in coronary blood flow produced by intravenous injections of thyroxin. Following operation, observations were made on successive days to establish control values. When this had been done, an injection of 1 mgm. of thyroxin for each kilogram of body weight was given intravenously. In one experiment a second injection was given on the following day. There was no significant change twenty-four hours after the injection, but within forty-eight hours the blood flow had markedly increased, having in two experiments reached the maximum, which was 230 and 244 per cent, respectively, above normal. The accidental death of one of these animals terminated the experiment. The coronary blood flow of the other had fallen from 244 per cent above the control flow at forty-eight hours to 61 per cent ninety-six hours after the injection of thyroxin. In a third animal the maximal flow, 142 per cent above normal,

TABLE 1
Effect of thyroxin on coronary blood flow

DOG	WEIGHT	CONTROL BLOOD FLOW	HOURS AFTER GIVING THYROXIN	INCREASE IN BLOOD FLOW
	<i>kgm.</i>	<i>cc. per minute</i>		<i>per cent</i>
11	18.2	70 to 78	48	230
12	17	112 to 127	24	0
			48	56
			72	100
			96	142
			120	36
13	15	80 to 83	24	17
			48	244
			96	61

was not reached until ninety-six hours after the injection of thyroxin, and in 120 hours the flow was about 36 per cent above the control values.

It is therefore evident that thyroxin causes a marked increase in the blood flow through the coronary arteries which is comparable to the augmentation observed in the femoral artery in response to this drug (table 1).

Effect of digestion on coronary blood flow. In a previous publication (4) we have reported that during digestion the blood flow in the somatic as well as the visceral vessels is increased from 35 to 127 per cent in response to the digestion of a mixed meal. Consequently it was to be expected that the additional work thrown on the heart during digestion would be reflected in an increased flow through the coronary arteries, and such is the case. We have observed an increase of as much as 84 per cent in the coronary blood flow of dogs during the digestion of food.

Effect of exercise on coronary blood flow. The influence of exercise on the coronary blood flow has been considered by physiologists for many years. Many calculations have been made as to the requirements of the heart during strenuous exertion. A series of experiments has been performed in order to obtain information on this question.

For investigations of this nature dogs are trained to walk on a treadmill, the belt of which is driven by a motor. When the belt is moving in a horizontal position it is necessary for the animal to walk rapidly and moderate exercise is thus required. The belt can be set at any desired angle from the horizontal to about 30° . When set at an angle of 15° , a dog weighing 15 kgm. performs work amounting to approximately 20 foot pounds per

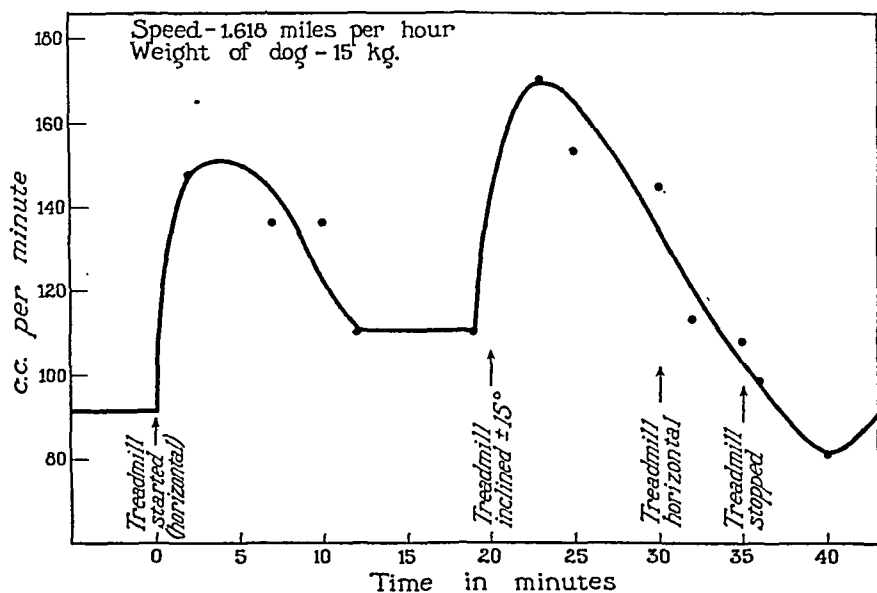


Fig. 1. Bloodflow in circumflex branch of left coronary artery before and during exercise on treadmill.

second. The speed of the treadmill can be varied from 1.152 to 3.756 miles per hour. We have routinely run the machine at a speed of 1.618 miles per hour.

In each experiment the coronary blood flow was observed first while the dog was resting quietly on the table, then while standing on the treadmill, and finally while the mill was running in a horizontal position or at an angle of 15° . There was not a great difference between the values for blood flow whether the animal was standing quietly or was lying quietly. However, on starting the treadmill there was an immediate rise in coronary flow which attained maximal values in about five minutes after which it gradually subsided to a much lower level in the succeeding five minutes.

The flow remained relatively steady at the lower level until the amount of exercise was increased. This was easily accomplished by rapidly elevating the treadmill to the desired angle, whereupon the flow again rapidly increased for about five minutes, when it declined to a lower level but remained above the second level reached during the previous increment of exercise. If the treadmill was then lowered to the horizontal, the flow rapidly diminished until it reached the second level about which it fluctuated. When the mill was stopped, the flow rapidly returned to the control level or even fell below the control values before stabilizing. Although every experiment did not reproduce the foregoing description in all details, nevertheless each experiment was comparable in the essential features to the one just described (fig. 1).

Coronary blood flow and weight of the heart. Various figures are given for the amount of coronary blood flow for each 100 grams of heart weight, but in general it should be understood that such data are of questionable value unless the conditions under which they were obtained and the physical state of the animal are indicated.

As has been stated, all the observations reported in this paper were made on the flow in the circumflex branch of the left coronary artery. According to Anrep, Blalock and Hammouda, the flow in this vessel represents about 50 per cent of the total inflow by way of the coronary arteries. When the total coronary flow is taken as twice the flow in the left circumflex artery and a calculation is made in terms of 100 grams of heart weight, there is no consistent relation seen in our data since, expressed in this manner, the values vary from 76 to 176 cc. All of the values for blood flow used in the calculations were obtained while the animals were lying quietly on the table.

Heart rate and coronary flow. Investigators have been divided on the question of the relation of heart rate to coronary flow. In our experiments we have not obtained any convincing evidence that there is a consistent relationship between pulse rate and coronary flow. The pulse rate may vary within wide limits while the coronary flow remains relatively constant (dog 10, protocol 1).

SUMMARY AND CONCLUSIONS

By the use of the thermostromuhr of Rein, experiments have been made on the blood flow in the circumflex branch of the left coronary artery of the intact dog. Observations have been made over periods as long as fourteen days.

Epinephrine causes a transient but marked increase in coronary blood flow amounting in some experiments to as much as four or five times the control values. The coronary blood flow is doubled by administration of nitroglycerine or amyl nitrite, but the effect is of short duration. In response to appropriate doses of thyroxin, increases in coronary flow as

great as 244 per cent above the control values were observed forty-eight to ninety-six hours after injection.

During the digestion of a meat meal the coronary flow was increased to a degree comparable to what has been observed in other vessels of the body. Exercise on a treadmill produced an initial rapid augmentation in coronary flow, which declined to a lower level as the exercise continued but additional work induced by changing the angle of the treadmill again caused a temporary increase in coronary flow, which declined to a lower value as the same degree of exercise continued.

A significant correlation between heart weight and coronary flow or between pulse rate and coronary flow was not found.

REFERENCES

- (1) ANREP, G. V., A. BLALOCK AND M. HAMMOUDA. *J. Physiol.* **67**: 87, 1929.
- (2) HOCHREIN, M. AND J. KELLER. *Arch. f. exper. Path. u. Pharmacol.* **159**: 300, 312, 1931.
- (3) HERRICK, J. F., H. E. ESSEX, E. J. BALDES AND F. C. MANN. *This Journal* **105**: 434, 1933.
- (4) HERRICK, J. F., H. E. ESSEX, E. J. BALDES AND F. C. MANN. *This Journal* **108**: 621, 1934.
- (5) REIN, H. *Ztschr. f. Biol.* **92**: 101; 115, 1931.

CAROTENE AND ASSOCIATED PIGMENTS IN MEDULLATED NERVE

JOHN PAUL BARTZ AND FRANCIS O. SCHMITT

From the Department of Zoology, Washington University, St. Louis, Mo.

Received for publication June 22, 1936

It has long been known that the yellow pigmentation of the normal vertebrate nervous system is due primarily to the lipochromes (Meschede, 1872; Mayer, 1876; Rosin, 1896; Neumann, 1909; Dolley and Guthrie, 1918). With the discovery that beta carotene is provitamin A this pigment assumed new importance in metabolic processes, and in view of its wide distribution in nervous tissue it is desirable that its rôle in nerve processes be studied. Its possible rôle as an oxygen transferring substance was suggested by Arnaud (1889) and Willstätter and Miegs (1907). That this suggestion may be of importance in connection with oxidative processes in nerve has been pointed out by Monaghan and Schmitt (1931). Schreiber (1932) discussed the rôle of chromolipid pigments in the ganglia of gasteropods. It has also been shown recently by Wald (1935) that carotene and vitamin A, in combination with certain proteins, together with the pigment retinene, are essential in chemical processes taking place in the rods of the retina.

Since nerve processes are most easily studied in cold-blooded peripheral nerve we have chosen the sciatic and brachial nerves of bullfrogs as material. In the present paper data are presented on the normal level of carotene and related pigments in these nerves, together with the effect of prolonged fasting and of feeding experiments.

METHOD. The combined sciatic and brachial nerves weighing about 1.5 gram obtained from three large bullfrogs sufficed for a single determination. The nerves were dissected free of extraneous tissue, weighed, minced, plunged into 20 cc. of 95 per cent ethyl alcohol, and allowed to stand in the refrigerator for 48 hours. The liquid was then decanted and to the residue were added 20 cc. saturated alcoholic potassium hydroxide and boiled for about five minutes. This results in complete solution of the tissue. After cooling, this liquid was combined with the alcoholic fraction and transferred to a separatory funnel. To this were added 200 cc. distilled water and 75 cc. ether and the mixture shaken. The carotenes, cryptoxanthin, xanthophyll and vitamin A collect in the ether layer. The water layer was drawn off and extracted with ether until no further color was removed.

The aqueous material was kept for vitamin G determinations. The combined ether fractions were repeatedly washed with water until the wash water was neutral to litmus. The ether fraction was then dehydrated with sodium sulphate and evaporated to dryness in a vacuum desiccator.

The various carotenoid pigments were separated as follows. The residue was dissolved in 25 cc. petroleum ether and washed repeatedly with 92 per cent methanol. The methanol fraction containing the xanthophyll was separated off. The petroleum ether fraction was then treated with

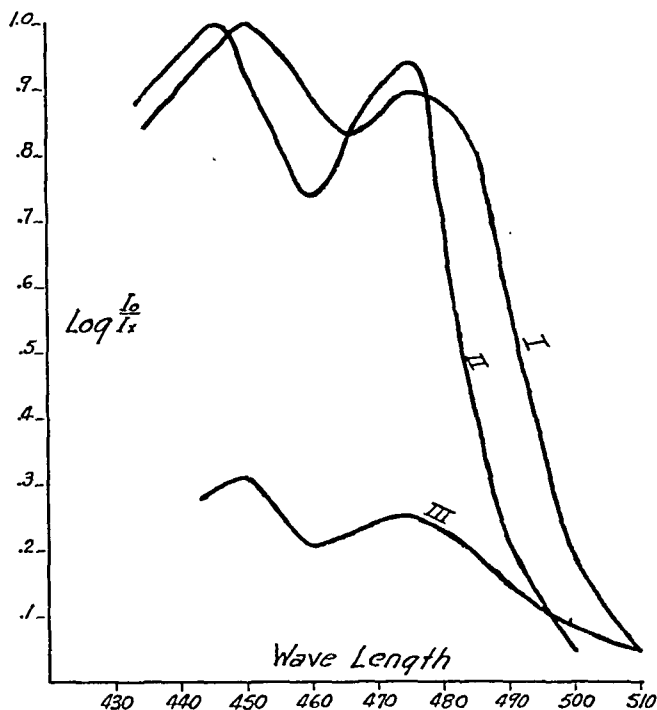


Fig. 1. Absorption curves of carotene in petroleum ether.

I—Beta carotene, 1.40 mgm. per liter; depth, 3 mm.

II—Alpha carotene, 1.40 mgm. per liter; depth, 3 mm.

III—Carotene in nerves; depth, 30 mm.

calcium carbonate, thus separating the carotene from the cryptoxanthin, the latter being adsorbed on the calcium carbonate. This fraction after elution was determined qualitatively by its absorption spectrum.

The carotene and xanthophyll were determined colorimetrically against standards freshly made from the pure compounds.¹ The carotene determinations were also checked spectrophotometrically.² A typical curve for

¹ Pure α and β carotene were obtained from the SMA Corporation. Xanthophyll was obtained from American Chlorophyll Company.

² We are greatly indebted to the Ralston Purina Company for the use of their Bausch and Lomb spectrophotometer.

nerve carotene is shown in figure 1, along with curves of pure alpha and beta carotene for comparison. Nerve carotene appears to contain a mixture of the two forms. The maxima of the nerve preparation were not as distinct as those from pure carotene but lay essentially in the same general region. The values obtained from the extinction coefficients agreed with those from the colorimetric method within about 0.5 gamma per gram. Vitamin A was determined colorimetrically by a modified Carr Price method.

RESULTS. For physiological experiments on frog nerves the animals are commonly kept in the ranarium often for a period of weeks or months

TABLE 1

Carotene and associated pigments in nerves of fasting bullfrogs

NUMBER OF BULLFROGS EXAMINED	TEMPERATURE OF RANARIUM	PERIOD OF FASTING	WEIGHT OF NERVE	CAROTENE	VITAMIN A	OTHER PIGMENTS FOUND
	°C.]	days	grams	γ per gram	units per gram	
8 (controls)	15	0	3.01	13.9	21	21 γ per gram Xanthophyll Cryptoxanthin
3	15	22	1.23	6.9	8	Xanthophyll
3	15	36	1.40	1.3	<2	Xanthophyll
12 (controls)	15	0	3.90	8.3	25	Xanthophyll
4	15	16	1.53	1.8	<2	Xanthophyll
4	15	22	1.42	1.6	<2	None
2	15	43	0.85	0.5	None	None
12	25	0	2.7	11.6	Present	Xanthophyll
4	25	0	1.8	9.6	Present	Xanthophyll
3	25	30	1.3	0.5	None	None
3	25	40	1.66	0.5	None	None

without feeding. Aside from determining the level of these pigments in the nerves of normal frogs it was, therefore, also desirable to obtain information on the changes in these pigments and in vitamin A which might result from such captivity. Each fresh shipment of Louisiana bullfrogs was kept in a separate ranarium. Eight to twelve frogs were killed immediately upon arrival and of the remainder, groups of three or four frogs were killed at various intervals of time later. In two series of experiments the fasting frogs were kept under "winter" conditions, the temperature of the ranarium being about 15°C. In one series the frogs were artificially "summered," the temperature of the ranarium being about 25°C.

The results are summarized in table 1. Obviously even under "winter"

conditions the carotenoid pigments and vitamin A are greatly depleted in three or four weeks of captivity without feeding. This depletion appears to be accentuated when the frogs are kept under "summer" conditions. This was also indicated by changes in skin color. The data in table 1 do not reveal the extremes between which the pigmentation varied because of the necessity of using the combined nerves of a number of frogs for each determination. The nerves of one frog may have been quite yellow while those of the next may have been practically white. An upper limit for the carotene level of the nerves of freshly caught frogs appears to be about 30 gammas per gram.

It has long been known that while the nerves of frogs kept for long periods in the ranarium without feeding are capable of conducting the impulse in fairly normal manner, abnormalities may be demonstrated.

TABLE 2

The effect of carotene and xanthophyll feeding on nerve pigmentation

NUMBER OF BULLFROGS EXAMINED	FASTING PERIOD PRIOR TO FEEDING TEST	FEEDING PERIOD	PIGMENT FED DAILY	WEIGHT OF NERVES	CAROTENE	VITAMIN A
	<i>days</i>	<i>days</i>	<i>mgm.</i>	<i>grams</i>	<i>γ per gram</i>	<i>units per gram</i>
3	43	0	0	1.3	None	None
4	45	7*	0.5 carotene	1.80	5.7	22
2	50	7	1.0 carotene	0.92	9.2	Present
			0.5 carotene			
2	45	12	+	0.83	0.5**	40
			0.5 xanthophyll			

* Tests made 8 days after last feeding.

** 0.5 γ per gram of xanthophyll found.

Thus Froehlich (1903) found that unless such kept frogs are fed, complete recovery of the action potential following readmission of oxygen after an asphyxial period is impossible. The present experiments demonstrate the serious depletion of carotene and vitamin A in the nerves of animals kept for extended periods without food. It seems obvious that nerve physiologists should give more attention to the diet of the frogs used in their experiments, particularly in the case of bullfrogs which, because of their hardiness, are frequently kept for months in the ranarium before being used in experiments.

Particularly with a view to further experiments on the physiological rôle of these pigments in which it would be desirable not only to deplete the nerves of pigment but also to increase the pigment to or above the normal level, frogs were fed quantities of carotene and of xanthophyll for a period of a week or so and the storage in the nerves determined. It will be seen

from table 2 that considerable storage of carotene occurred in animals which had been completely depleted by a preliminary period of fasting. In some of these animals the nerves were deep orange in color. It is significant that of the group fed a mixture of carotene and xanthophyll the pigment was apparently converted largely to vitamin A rather than being stored as such. The rôle of xanthophyll in this interconversion is not clear.

SUMMARY

1. The carotene and vitamin A content of the peripheral nerves of bullfrogs has been determined.

2. The level of these pigments in nerves of frogs kept in the ranarium without food drops considerably and may be entirely depleted in three to four weeks depending on the temperature.

3. Daily feeding of carotene causes considerable storage in the nerves within a week. If xanthophyll is also fed there is considerable conversion to vitamin A in the nerves.

REFERENCES

- ARNAUD, A. *Compt. Rend. Soc. Biol.* **109**: 911, 1889.
DOLLEY, D. AND J. GUTHRIE. *J. Med. Res.* **39**: 123, 1918.
FRÖHLICH, F. W. *Ztschr. f. allg. Physiol.* **3**: 131, 1903.
MAYER, G. *Arch. f. Psych. u. Nervenkrankh.* **6**: 1876.
MESCHÉDE, F. *Virchow's Arch.* **34**: 249, 1865; **54**: 100, 1872.
MONAGHAN, B. AND F. O. SCHMITT. *Proc. Soc. Exper. Biol. and Med.* **27**: 705, 1931.
NEUMANN, E. *Virchow's Arch.* **197**: 39, 1909.
ROSIN, H. *Deutsch. Med. Wchnschr.* **22**: 495, 1896.
SCHREIBER, G. *Biol. Abst.* **6**: 3980, 1932; **7**: 5599, 1933.
WALD, G. *J. Gen. Physiol.* **19**: 351, 1935.
WILLSTÄTTER, R. AND MIEGS. *Ann.* **355**: 1, 1907.

GERMINAL RESPONSE (IN MALE MICE) TO ENVIRONMENTAL CONDITIONS¹

CORDELIA L. OGLE

From the Laboratory for Experimental Medicine, University of Cincinnati

Received for publication June 22, 1936

Statistical data have been presented by Mills which show that human fertility in any given population is highest between 40°F. and 65°F. and that it declines sharply at mean temperatures above 70°F. or below 40°F. (1), (2). He has also shown that sexual maturity has an earlier onset in the human female in the more invigorating zones than in those less stimulating (3). Results comparable in all essentials to the human data have been obtained in this laboratory with albino mice (4). Female descendants of mothers kept under stimulating environmental conditions bore young sixteen days earlier than did their mothers. Coincidentally, it was found that the fertility of male mice, and also the position of the testes, were influenced by the climatic environment. Conditions of steady moist heat permitted only a very low degree of fecundity, although the testes rested in large loose scrotal sacs. On the other hand, retention of the testes intra-abdominally did not produce sterility under invigorating environmental conditions. These findings, obtained by matings, have been augmented by histological studies which reveal a morphological basis for this very marked dissimilarity of sexual functionings in animals exposed to different environmental conditions.

EXPERIMENTAL CONDITIONS AND RESULTS. The data which follow were obtained by examination of the testes of mice from the experimental groups previously described from this laboratory (4).² Mating results

¹ This work, carried on during 1933 and 1934, was aided financially by the National Research Council and the Ella Sachs Plotz Foundation. I wish to take this opportunity to express my appreciation to these donors for their aid.

Data herein presented were taken from a Ph.D. thesis, University of Cincinnati, 1934.

² The experimental groups consisted of control room group, a cold room group, a hot room group and two groups of animals that were shifted daily between the hot and cold rooms. For convenience, these shifted groups are considered together here because their reactions to great temperature variability were alike. The control room was kept at 70-80°F. The cold room had a temperature range of 60-68°F. and in the hot room the temperature ranged from 88-92°F. with the humidity near 75 per cent. The construction of these constant temperature rooms has been described elsewhere (5).

TABLE 1

1. At 8 weeks of age	
Cold room males (13):	Spermatozoa present, many very active tubules, pyknosis, peripheral tubules vacuolated, no giant cells
Hot room males (12):	Spermatozoa present, lumina large, peripheral vacuolization, pyknosis, giant cells rarely observed.
2. At 12 weeks of age	
Cold room males (13):	Same as at 8 weeks
Hot room males (15):	Spermatozoa present, varying stages of activity, peripheral tubules vacuolated, giant cells more frequent
3. At 16 weeks of age	
Cold room males (9):	Spermatozoa present, fairly active but thin epithelium, pyknosis, giant cells rarely observed
Hot room males (3):	Spermatozoa present, thin epithelial linings, increase in giant cells, extensive vacuolization of peripheral tubules
Change group males (8):	Spermatozoa present, varying stages of activity, peripheral tubules vacuolated, giant cells occasionally observed, small tubules
4. At 20 weeks of age	
Cold room males (16):	Spermatozoa present, epithelial linings active but thin, peripheral tubules vacuolated, pyknosis
Hot room males (9):	Few, if any, spermatozoa; more advanced degeneration, giant cells numerous
Change group males (10):	Spermatozoa present, thin epithelial linings, varying stages of proliferation, peripheral tubules vacuolated, few giant cells, small tubules
5. At 25 weeks of age	
Control room males (6):	<i>Tubules normal and highly active.</i>
Hot room males (5):	Some moderately active tubules with spermatozoa present, interstitial tissue very prominent, peripheral tubules vacuolated
Change group males (10):	Few spermatozoa, peripheral tubules vacuolated, giant cells frequent, small tubules
6. At 31 weeks of age	
Change group males (9):	Tubules very small, extensive degeneration, giant cells frequent, tubular shrinkage
7. At 38-52 weeks of age	
Hot room males (5):	Same as at 25 weeks
Cold room males (3):	Same as at 20 weeks
Control room males (8):	Same as at 25 weeks
Change group males (6):	Same as at 31 weeks

showed that the cold room males were equally as fertile as control room males although the testes of the former resided within the abdominal cavity so long as the animals were kept in the cold room, while on the contrary the sex glands of the hot room and change group males rested in large loose scrotal sacs and these animals had a very low fertility.

Comparable numbers of animals from each group were sacrificed at similar ages. The animals were killed quickly either by a blow on the head or by ether. The testes were studied histologically after fixation in 4 per cent formaldehyde and staining with hematoxylin and eosin.

A brief summary of the microscopic findings in the four groups is presented in table 1.

Nuclear material was pyknotic in all tubules except those of the control room mice kept constantly in the control room. Also the cytoplasm of the control room animals stained more deeply with eosin, under the same technique, than did that from any other group of mice. The hot room and cold room males presented similar testicular tubules up to the age of twelve weeks; most of the peripheral tubules were vacuolated, nuclear material was pyknotic and varying degrees of proliferation were observed (figs. 1 and 2). From this age on the hot room males showed more extensive vacuolization, the tubules had thin epithelial linings and contained a large number of giant cells. Such degeneration is illustrated by figure 1, which portrays a cross-section of the testes from a hot room male at 20 weeks of age. Between the ages of eight to twelve months, giant cells were not so abundant, yet many tubules were bare, the interstitial tissue very prominent and the degree of activity quite variable. Spermatozoa were present in some tubules but not usually so where there was extensive vacuolization.

The germinating epithelium of the cold room group males was usually thin, but many spermatozoa were present. Giant cells were rarely observed. Nine cold room males (five months of age) were placed in the hot room, kept there for varying periods and then killed. Within three days after transfer to the hot room, the testes of these mice had descended into deep scrotal sacs. Those animals, originally from the cold room, which were killed after being in the hot room for one month, presented a highly degenerated germinal tissue with extensive vacuolization, many giant cells and few, if any, spermatozoa (fig. 4). Following a sojourn of two months in the hot room there were fewer giant cells and an increase in all types of germinal cells save spermatozoa. Hence, animals transferred from an invigorating environment to one of humid heat suffer an acute loss in germinating epithelium soon after their change in climate. In due course these transferred animals become adapted to their new environment and the tubular linings are partially regenerated, but mature germinal cells are not produced. This regeneration is complete and more rapid when the

Fig. 1



Fig. 2



Fig. 5



Fig. 6

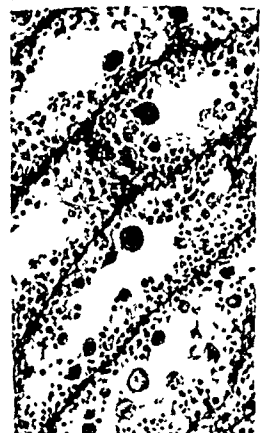


Fig. 3

Fig. 4

Fig. 7

Fig. 1. Hot room male no. 501. Age 9 weeks. Body weight 16.0 grams. (200X.)

Fig. 2. Cold room male no. 621. Age 8 weeks. Body weight 23.3 grams. (200X.)

Fig. 3. Hot room male no. 43. Age 20 weeks. Body weight 17.0 grams. (200X.)

Fig. 4. Cold room male no. 117. Killed after 36 days in the hot room, at which time the animal was 6 months old. Body weight was 26.3 grams when transferred to the hot room, and 23.3 when killed. Much hair was lost from the abdomen. (200X.)

Fig. 5. Cold room male no. 101. This animal was placed in the hot room for 26 days, and returned to the cold room for one week before being killed. Age at death 6 months. (200X.)

Fig. 6. Control room male no. 238-9. Age nine months. Placed in hot room for 10 days and killed upon removal therefrom. Body weight when removed from control room was 28.9 grams; 27.9 grams at time of death. (200X.)

Fig. 7. Change I group. Male no. 229. Weight 25.2 grams. Age at death 6 months. (200X.)

animals are returned to their original habitat. One cold room male was placed in the hot room for twenty-six days and at the end of this period was returned to the cold room for one week before being killed. In spite of the fact that the testes promptly returned to their abdominal position when the animal was replaced in the cold room, regeneration occurred soon. The germinal epithelium was fairly thick, although it was almost devoid of spermatozoa and contained a few giant cells (fig. 5).

The control room males, when placed in the hot room, reacted in the same manner as did those of the cold room so treated. Ten days in the hot room was the shortest exposure of the control room or cold room males to the humid heat. At the end of this time, the germinal tissue has undergone extensive degeneration, and contained a great number of giant cells. No spermatozoa were present (fig. 6).

The testes of the males shifted daily between the hot and cold rooms showed various degree of activity, with occasional giant cells, up until the age of approximately six months. At this time most of the tubules showed much degeneration with few spermatozoa, many giant cells, and prominent interstitial tissue (fig. 7). The tubules were quite small in all of the animals of this group. We see here in the degenerated germinal tissue the reason for so very few pregnancies resulting from the mating together of males and females that were shifted daily between the hot and cold rooms. These males, and those of the hot room, possess scant amounts of actively proliferating germinal epithelium, while a most intense loss of this tissue occurs quickly after the transfer of control or cold room mice to the hot room.

Discussion. These findings provide strong presumptive evidence that the physical environment markedly influences the histology, as well as the activity, of the germinal epithelium of the testes. In every instance, exposure to humid heat led to the degeneration of this tissue, accompanied by the production of many giant cells. Large, loose scrotal sacs, such as those possessed by the hot room and shifted males, are considered by Sundstroem as an adaptation to facilitate heat loss by radiation (6). Moore and co-workers (7) and Fukui (8) found that when the testes were kept at the temperature of the peritoneal cavity, the germinating epithelium was rapidly destroyed. In Moore's experiments, restoration of the germinal epithelium occurred only when the testicular temperature was lowered a few degrees below that of the body. In all mammals examined by him and Quick (9), the scrotal temperature was below that of the peritoneal cavity. In the experiment here recorded the cold room mice retained their testes intra-abdominally and were fully as fertile as control ones. Transfer of cold room or control room mice to the hot room led to an early epithelial degeneration, even though the testes of the former group of animals descended into scrotal sacs while they were in the hot room.

However, a prolonged stay in the hot room led to the regeneration of all types of germinal cells except spermatozoa. Such regeneration was accomplished even more quickly by the return of these transferred animals to their original habitat, where mature germinal cells also were produced.

The experimental conditions under which these animals were kept affected primarily the ease of body heat loss. Body heat production is definitely suppressed under conditions of humid warmth, and this suppression is probably based on a decline in function of the adrenal cortex. It is not advisable to enter here into a review of the literature showing a close relationship to exist between adrenal and sex gland functionings. In general it has been well shown that the two sets of glands are intimately linked. Hence it is in no way surprising that we should find suppression of fertility and gonadal activity by environmental conditions that render difficult the loss of body heat.

In the human being, sudden exposure to humid heat frequently leads to functional suppression of the adrenal gland (10), while it has also been shown with experimental animals that the metabolic rate and heat production decrease as the temperature of the environment is raised (11). Hence, under the conditions of this work, it seems probable that the state of activity of the germinating epithelium is more intimately dependent upon the animal's metabolic level than upon the anatomical position of the testes. This point is clearly illustrated in the immediate desquamation of the spermatid tubules of animals suddenly exposed to humid heat, since the desquamation takes place in spite of a migration of the testes from their intra-abdominal position down into deep scrotal sacs.

The suppression in the metabolism of the germinal tissue affects both the nuclear and cytoplasmic materials. Mature spermatozoa, the most labile products of this epithelium, rapidly disappear, but the nuclear material of the younger germinal cells continues to divide long after the cytoplasm ceases to do so, and thereby results in the production of multinuclear masses of protoplasm. This germinal suppression seems most probably associated with a general metabolic subsidence as the external humid heat makes body heat loss difficult. The close linkage of adrenal cortical activity to heat production in the body and also to activity of the sex glands, intensifies the responsiveness of these glands to external temperature conditions.

SUMMARY

1. The germinating epithelium of male mice kept under conditions of constant humid heat undergoes degeneration, accompanied by giant cell formation. Few actively proliferating tubules are present between the ages of four and twelve months, although the testes reside in deeply pendent scrotal sacs.

2. Mice adapted to an invigorating environment retain their testes

intra-abdominally and have active germinal tissue. Submission of such males to humid heat leads to an abrupt degeneration of the testes. Control mice show a similar reaction to humid heat.

3. After exposure to humid heat, the germinal tissue of cold room or control room males is regenerated more rapidly when these animals are returned to their original experimental climate than when kept for prolonged periods under the heat conditions.

4. Microscopic studies of the testes verify the fertility findings previously reported, viz., that the sexual functions are quickly and markedly affected by changes in the stimulating character of the environment. Moist heat, by rendering body heat loss difficult, produces a prompt suppression of fertility and of microscopic evidences of germinal activity.

REFERENCES

- (1) MILLS, C. A. *Ohio J. Sci.* **30**: 256, 1930.
- (2) MILLS, C. A. AND F. A. SENIOR. *Arch. Int. Med.* **46**: 921, 1930.
- (3) MILLS, C. A. *Am. J. Hyg.* **15**: 593, 1932.
- (4) OGLE, C. *This Journal* **107**: 628, 1934.
- (5) OGLE, C. AND C. A. MILLS. *This Journal* **103**: 606, 1933.
- (6) SUNDSTROEM, E. S. *This Journal* **60**: 397, 1922; *Physiol. Rev.* **7**: 320, 1927.
- (7) MOORE, C. R. AND R. OSLUND. *Anat. Rec.* **25-26**: 343, 1923.
MOORE, C. R. *Am. J. Anat.* **34**: 337, 1924-25.
- (8) FUKUI, N. *Japan Med. World* **3**: 160, 1923.
- (9) MOORE, C. R. AND W. J. QUICK. *Anat. Rec.* **25-26**: 344, 1923.
- (10) MILLS, C. A. *Arch. Int. Med.* **42**: 390, 1928.
- (11) GELINEO, S. AND J. GIAJA. *Srpska kralavska akademija Belgrade* (French title: *Académie des sciences de Serbie*) **98**: 115, 1933.

COMPONENTS OF THE ELECTRICAL RESPONSE OF THE OPTIC CORTEX OF THE RABBIT¹

G. H. BISHOP AND JAMES O'LEARY

From the Laboratory of Neurophysiology and Department of Anatomy, Washington University Medical School, St. Louis, Mo.

Received for publication June 25, 1936

This paper deals with the form of the electrical responses of the optic cortex to stimulation of the optic nerve, as recorded from different depths in the cortex. Histological material from the animals upon which the experiments were performed is being further studied, with a view to correlating the electrical responses obtained with the structure at the specific levels led from. It is to be presumed *a priori* that the spatial sequence of elements successively activated in the cortex will not be simply linear, and that the temporal sequence of their responses will not be a simple succession. In spite of this, a first approach seems feasible through considering the cortex as a modified peripheral nerve trunk, in which certain elements may terminate, and others originate, between any two electrodes placed along the pathway of the impulse. This "nerve" will have different effective cross-sections at different levels, and complicated shunts of extraneous tissue.

To make the conditions as simple as possible, the rabbit was chosen as experimental material because its cortex is of the lissencephalic type, that is, without fissures. The optic nerve was stimulated directly, after removal of the eye, by single electric shocks or 2 ms. galvanic currents, each stimulus resulting in a single volley of impulses in parallel fibers passing over the pathway as nearly synchronously as possible. Light ether anesthesia was employed. The pathway then consists of optic nerve and tract (mostly crossed in the rabbit chiasma), the dorsal nucleus of the lateral geniculate body, the optic radiation, a sequence of cellular elements situated in the layers of the cortex, and finally the corticofugal fibers returning from the cortex through the optic radiation. The preparation and manipulation of the animals have been described in previous publications (Bartley and Bishop, 1933).

Most physiological recording from the cortex has dealt with its delimitation into cyto-architectonic fields. The response of any one field or loca-

¹ Aided by a grant (to G. H. Bishop) for research in neurophysiology from the Rockefeller Foundation.

tion must be extremely complex, and its record must be the sum of many different responses of elements in different layers or positions in the cortex. It may be expected, from the degree of anatomical stratification exhibited, that a vertical differentiation should be quite as specific as a horizontal one, although, as emphasized by Lorente de N6 (1934a), the too obvious interpretation of the stratification of the cortex may lead to a deceptively simple concept of the functional relationships of the elements concerned. To the extent, however, that one stratum contains elements that are alike, and to the extent that they can be *activated synchronously*, such a sheet of elements will be comparable to a segment of peripheral nerve occupied by a volley of impulses. Electrodes in contact with this stratum, above and below a given level, need not be in contact with the same identical elements to give a valid record of such elements, but need only be in contact with similar elements active at the same time.

TECHNIC. In general, only those records are available for analysis in which the response to stimulation is large relative to the spontaneous activity occurring. The usual cortical responses of rabbits under light ether anesthesia are of the order of one millivolt or more, but significant elements of the responses may be only one-tenth of this, and less than the level of spontaneous cortical oscillations. Maximal single stimuli have usually been employed, at intervals of one second, two or more series of several successive shocks each being delivered for each strip of record. Electrodes are placed on the surface, and at various depths in the cortex, optic radiation, etc., and different pairs employed subtending different strata. The stimulating electrodes are not grounded, the rabbit rests on an insulated table, the single ground connection is usually the deeper of two electrodes, and negativity at this electrode gives an upward deflection in the record. After each experiment the carotid is injected toward the head with alcohol-bichloride fixative, the veins opened, the brain then removed to fixative and the significant parts finally sectioned and stained to locate the electrode positions with respect to the cortical structure. Usually a small blood clot locates the path of a needle electrode, otherwise tissue distortion is found. The surface electrodes consist of fine steel sewing needles (no. 10) with points filed off, the ends embedded in a block of celluloid cement 2 to 3 mm. in diameter whose flat surface penetrated by the needle distributes its weight. The deep electrodes are similar needles with points, coated with insulating cement to within 1 mm. of the tip. They are thrust as tangentially as convenient to the desired depths, and are unsupported except by their contact with the cortex, being free to move with the movements of the exposed brain accompanying respiration. Fine flexible wires connect each electrode to corresponding points of each of two selector switches, one of which leads to the ground of the amplifier, the other to the grid. Records are taken on 60 mm. bromide paper, drawn across and in

contact with the face of the oscillograph, whose beam moves only vertically. Another oscillograph in parallel to this but with time axis deflection presents the record visually. The start of this time deflection causes a disturbance that is recorded as a shock-like deflection preceding the stimulus artefact, which marks 1-second periods on the record.

Considerations of interpretation. Several general propositions may be applied to the cortex treated from the point of view of the physiology of peripheral nerve trunks. 1. Insofar as elements of a given type (for instance, cells with short processes) lie effectively between such electrodes, it will not matter whether the electrodes are close to these elements or at some distance above and below, or whether exactly vertically over each other; such structures will behave as a sheet of elements, or a polarized layer, imposing whatever difference of potential they set up in their ac-

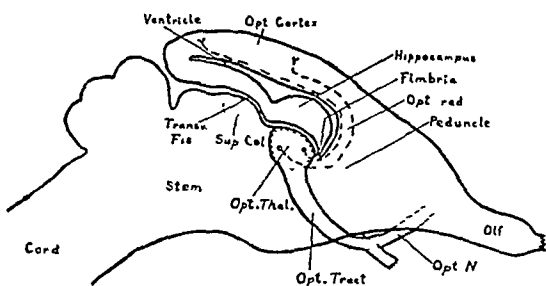


Fig. 1

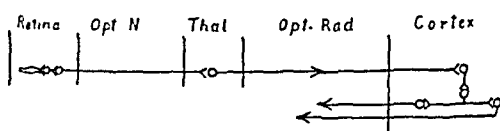


Fig. 2

Fig. 1. Diagrammatic projection of the optic pathway of the rabbit on a sagittal plane.

Fig. 2. Diagram of the optic pathway to compare with a linear stretch of peripheral nerve. Electrodes placed one above the other in the cortex will record impulses traveling in both directions, with complications of synapses between leads, etc., as discussed in text.

tivity on all the tissues adjacent. 2. Insofar, however, as such elements extend vertically through the cortex, the distance between leads along such structures will affect the diphasic record quite as in peripheral nerve trunks, and the amplitude and form as recorded will be a function of such separation of leads. 3. Further, if an element originates between leads, passing only the second, it will tend to give a monophasic (second phase) component of potential instead of a diphasic one; if it passes the first lead and terminates between leads, mainly a first phase will appear. For instance, since the number of cell axons leaving the cortex increases with depth in the cortex, the lower of two electrodes in the cortex will be in contact with a greater density of such axons than the upper, by the number which arise between electrodes, and with conduction downward in the cortex, the "second phase" of the summed diphasic potential assignable to them will be greater than the first. If the summation is such that individual responses

are not recognizable, but only the mass effect is recorded, the upper electrode will then be on the average less negative, that is, more positive, than the lower, and the net effect of conduction through such elements in the cortex may be a surface-positive potential wave, more or less monophasic, such as is usually found in cortical records. Finally, 4, since elements, or portions of elements lying parallel to the surface of the cortex, will in general run in random directions, their potentials will tend to annul each other, and only the vertical components of the potentials of such elements will affect the record. Any cortical record must therefore be an incomplete one.

The pathway of the visual impulse. To facilitate the consideration of the optic pathway as a modified peripheral nerve trunk, a diagrammatic sketch of its course may be presented (figs. 1 and 2). The fibers of the optic radiation pass downward and forward from the thalamus, in an arc, then upward and posteriorly to the optic area. The region from which responses to optic nerve stimulation can be obtained is roughly oval. It extends from the posterior pole of the cortex for 12 to 15 mm., or half way to the olfactory bulb, and from slightly medial to a shallow sulcus (which lies, in the rabbit, about 4 mm. from and parallel to the midline), it extends laterally 10 to 12 mm. It corresponds fairly well with the area striata as defined by Rose (1931) except that the area we find responsive may overlap what Rose pictures as the parastriate; we find the active-inactive margin to vary by 2 mm. or more from rabbit to rabbit.

The afferent fibers entering the cortex, at least in the cat and man, do not pass completely through it, but terminate in the region of the internal granular layer, roughly in the middle of the cortex, corresponding to the stria of Gennari of such animals as show this definitely (Cajal, 1923; Poliak, 1932). The first cortical neurones to respond should not therefore have their synapses located above this level. The granular layer is a region of cells with axis cylinders which pass both upward and downward in the cortex, but many of which do not leave the cortex. Both above and below the internal granular layer are regions of pyramid cells (superficial and deep) whose axons run in general perpendicularly through the cortex toward the basal white matter. The impulse started in the optic nerve must therefore pass in general to the middle layers in a direction toward the surface of the cortex, then, after synapsing one or more times, proceed (at least in part) further toward the surface, and finally reverse in direction after further synapses and pass from surface to the interior.

The activities in these successive components of the pathway may be expected to be set off from each other, and to be therefore recognizable, by temporal delays at synapses, by differences in amplitude as recorded from different depths in the cortex, and possibly by polarity of the potential record. Further, as the mass impulse, starting as a synchronous activity in parallel axons of the optic nerve, passes along fibers having different

conduction rates, and across synapses having different accessibilities, the individual elements of the response will be progressively dispersed, the synchronous volley be modified to a scattered distribution in which the individual responses will overlap and be summated to form a longer-lasting potential elevation. The form of the earlier waves, less dispersed temporally, will tend to be determined by the form of the individual impulse in each similar element. The form of the later responses will be a function largely of the *temporal distribution* of the responses of the individual elements. Finally, if at some region a single response in each element sets up repetitive responses in following elements, the temporal distribution of responses, and thus the form of their summated effect, will be still further modified. The records of cortical potentials may be examined from the point of view of these propositions.

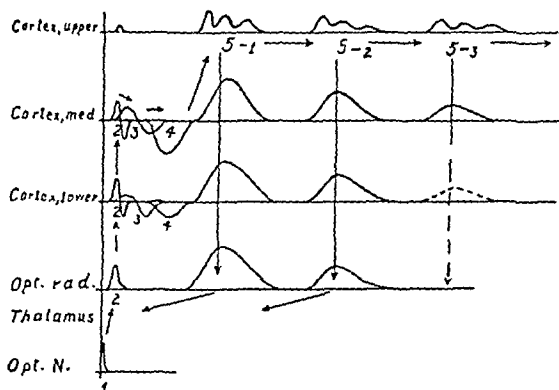


Fig. 3. Diagram of responses of the optic pathway. At least four elements of the response, following the response of the optic nerve, can be distinguished in some records, although any two adjacent elements, each presumably complex, may be confluent in a given record. The last of these four may be repeated several times at about $\frac{1}{2}$ second intervals following a single shock. There is a discharge of corticofugal fibers during at least the first of these repetitive cortical discharges, which appears to facilitate the thalamic neurones to a second discharge from the optic nerve.

Form of cortical response. The first potential observable following a single strong shock applied to the optic nerve (fig. 3, 2) is a narrow spike, typically diphasic, followed immediately by a second somewhat broader diphasic deflection (3). These two may be fused, to form a single diphasic wave, the first phase of which often passes off the record at a sensitivity suitable for recording subsequent events, or they may be fairly well separated from one another. The first of these must include whatever is recorded of the response of afferent fibers from the thalamus, and may also include responses of cortical elements. Following these occurs a long-lasting, surface-negative deflection (4), followed in turn by a long-lasting surface-positive deflection. The latter is usually the most prominent

feature of the cycle (5-1); it will be referred to as the main cortical response. It is accompanied by a discharge of corticofugal fibers in the optic radiation, and may be repeated (5-2, etc.) at about $\frac{1}{5}$ second intervals, but with decreasing amplitude, until it finally becomes undistinguishable above the spontaneous activity. The potentials between the first response and the main response are recorded quite differently from different depths of the cortex. In previous papers (Bartley and Bishop, 1933a, b) we have interpreted this main cortical response as the first repetition of the deflection immediately following the shock artefact. It is now obvious that it is one of a progressive series of events initiated by the first response, but not a duplicate of it, and is itself the first of the repetitive series. For reasons that will appear below we cannot say at present whether the events preceding this main discharge are also repeated, but they probably are.

These are the responses so far differentiated, and we do not suppose that they are simple. Each may be the summation of the responses of several different varieties of neurones. Their time relations are as follows, varying considerably in different animals, with the strength of stimulation, and with other conditions conveniently disposed of as the "state of the animal." The initial discharge commences as early as 3 to 5 ms. after the shock, its duration is uncertain because it is diphasic, but is short enough to suggest that each element responds only once to each shock, that is, the relation of optic nerve fiber response to optic radiation fiber response is one to one across the thalamic synapse (for single stimuli). The next cortical response commences either during the first phase of the initial discharge, or early in its second phase, 10 to 20 ms. after the shock. It also is diphasic, and is sometimes brief enough, allowing for temporal dispersion at synapses, to suggest that here also a one to one relation exists across the synapses. The negative deflection is much longer in duration; its start is usually confluent with the second phase of the previous potential, its duration 50 to 100 ms. It might represent either a summated and asynchronous repetitive discharge, a series of overlapping discharges in successive elements, or a "slow potential" in each element responding. The main surface-positive elevation, which is accompanied by the summed activity of corticofugal fibers in the optic radiation, may arise smoothly from the preceding negative wave, or be apparently separated from it; its start is difficult to measure, but its crest falls typically at about 180 ms. after the shock, and is repeated once or more, often with strikingly similar configuration, at $\frac{1}{5}$ to $\frac{1}{3}$ second intervals thereafter. Its duration may vary from 80 to 200 ms.

Both the form and the magnitude of each component of potential in a given animal are functions of the depth in the cortex from which the potentials are led off. From two electrodes, one on the surface and a second above the internal granular layer (in the superficial pyramids), the initial

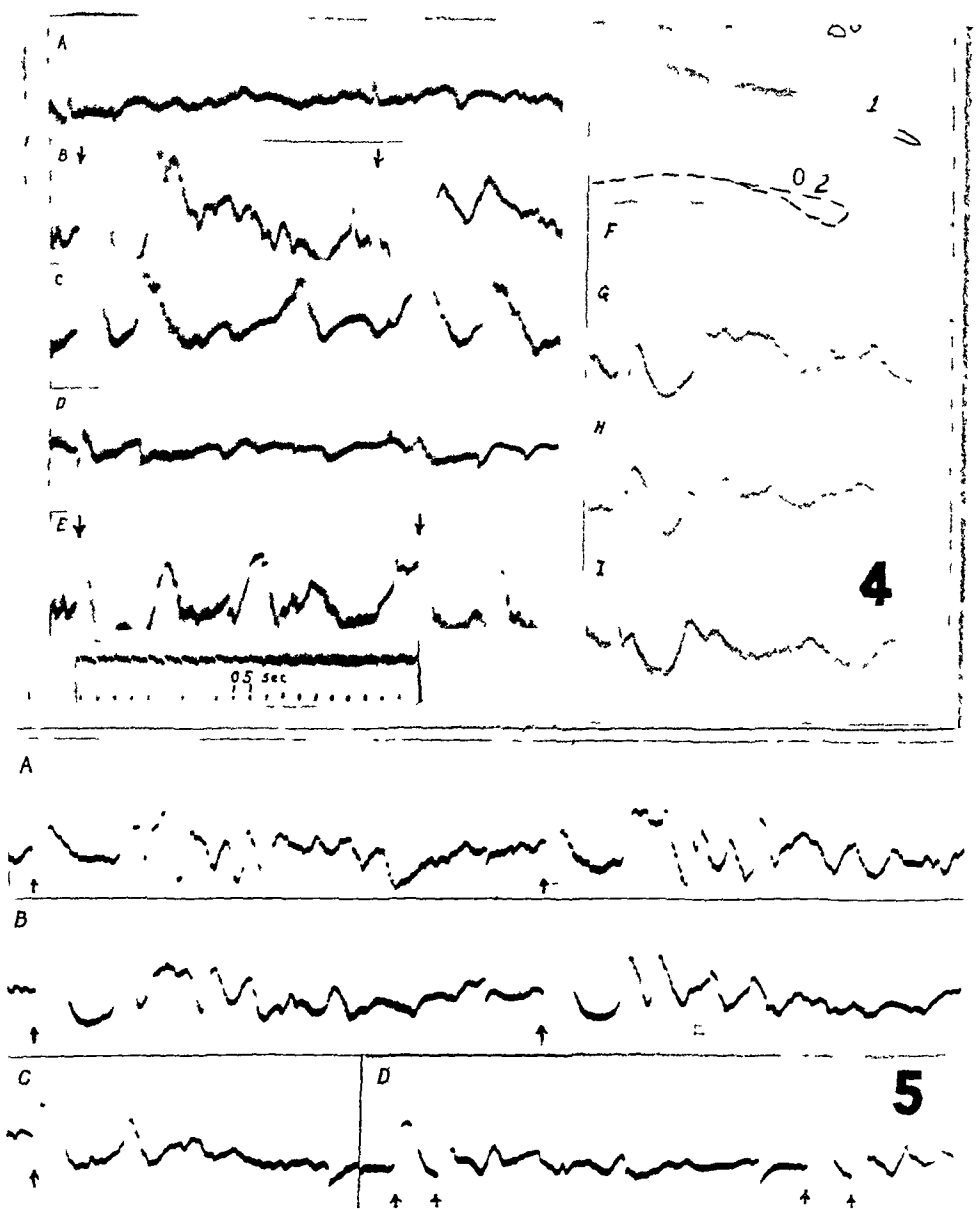


Fig. 4. Cortical records from electrodes whose position is shown in *F*, a section passing through the trace of needle 1. The circle locating the point of needle 2 is projected from another section. While 2 appears to be definitely below 1, it ran at a sharper angle, and its uninsulated point made contact with the cells above the fiber layer. Needles 1 and 2 are therefore effectively at the upper and lower margins of the sixth stratum of the cortex. *A*, spontaneous activity; *B*, leads 3 to 1; *C*, lead 3 to 2; *D*, leads 1 to 2; *E*, 1 to 2 at three times the sensitivity of recording apparatus. A second stimulus falls in a repetitive response to the first. *G*, *H*, *I*, responses to the first, second and eleventh stimuli of a series at 1 per second, leads at surface and below cortex. Same preparation as above, but after more than an hour's use. Each

discharge appears as a low, sometimes nearly monophasic, surface-positive spike. Led from electrodes deeper in the cortex, it is higher and distinctly diphasic, the first phase surface-positive (fig. 4, *H*). That is, the proximal (or ground, deep) electrode is first negative; the impulse is traveling toward the cortex surface; and most of the elements presumably end before they reach the surface. The cortical response following this is not usually recorded by leads subtending only the surface layers, and from deeper electrodes, its first phase seems to be more prominent from the shallower, its second from the deeper strata. Its origin would seem to be from elements in the internal granular and deep pyramidal layers. It is recorded not only from localized leads, but also when one lead is on the surface and the second at an indifferent point,² either below the cortex or elsewhere on the head (fig. 4, *G-I*).

The surface-negative wave following this is likewise absent or weak in superficial leads, present in deep leads; and this is recorded from leads across the whole cortex, but less sharply than from the middle layers. The main surface-positive wave is recorded at every level and from the optic radiation. It presumably includes the discharge of pyramidal cells, both superficial and deep. At any depth, and with any separation of electrodes, the deeper electrode is typically negative to the shallower, the

strip 1 second. The first two waves are well separated out, the third (downward deflection) is confluent with the second phase of the second. The fourth wave, at least in *G*, is set off by its abrupt rise from the third, and is repeated with characteristic form. Note falling off in amplitude and in complexity of the elevations with repetition, even at 1 per second.

Fig. 5. *A* and *B*, records of responses, 2 shocks each, at 1 second interval, at two different regions of the cortex. Electrodes at surface and below the cortex. Except for the shock distortion, which interferes with the first part of the record, successive responses of the same area vary as much as responses from two different regions. *C*, a record taken after some time, similar leads to *A* and *B*, showing simplification to a definite 5-per-second rhythm of a picture previously much more complex. *D*, two pairs of shocks, the second of each following the first by about 140 ms., and giving a smaller response whose waves alternate with those of the first shocks. That is, the first wave following the second shock is the main response to the first shock (compare *C*), the next wave is the main response to the second shock, etc.

² In a technical sense, to an impulse activating the whole optic cortex synchronously, no electrode is an *indifferent* one. Such an electrode, for instance, on the side of the head, is effectually in contact, through head and brain structures acting as an extension of that electrode, with the sheet of fibers composing the optic radiation underlying the optic cortex. The optic cortex is large enough in area so that, between an electrode on its surface, except near the margin, and one anywhere on the head, no significant shunting takes place. In confirmation of this view, there is no significant difference in the record of response to stimulation wherever the second electrode is placed, until it enters the optic cortex itself (or the optic radiation leading to it).

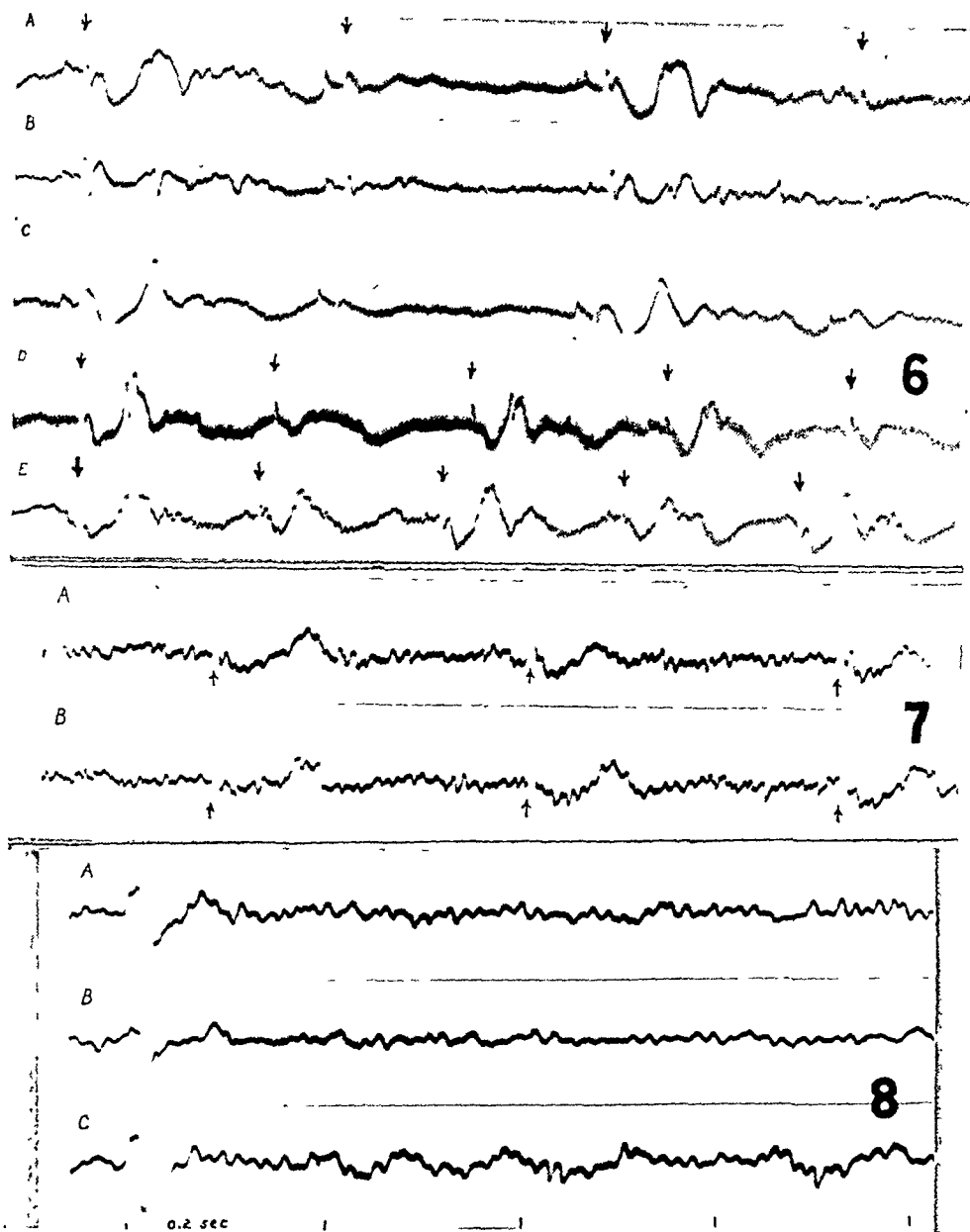


Fig. 6. Responses of the optic cortex to shocks at 1 second intervals applied to the optic nerve. With medium strength shocks (below maximal) only every alternate stimulus is effective (*A* to *C*). With stronger shocks, every one takes effect, but with alternations in amplitude (*E*). Still stronger shocks will in some rabbits each be equally effective. Note that when the shock is ineffective in causing a cortical response, even the first element of the response fails to occur. In *D*, at a strength between *A* and *E*, the second response fails to occur, but the fourth is present, the fifth being low.

A, one lead electrode on cortical surface, the second a needle whose uninsulated point lay below cortex. *B*, surface to granular layer, about middle of cortex. *C*,

amplitude of the wave being roughly a function of the distance between electrodes. Its configuration varies, sometimes having a smooth sine form, oftener consisting of a long elevation with ripples or definite peaks superposed on it, a common form being one main spike rising abruptly from the negative deflection, followed by a succession of 1 to 4 lesser spikes. Recorded from superficial layers it may consist only of such a series of apparently monophasic spikes, while from lower strata of the same preparation the spikes are nearly absent and only a long smooth elevation appears.

It is not uncommon for this elevation to vary during an experiment, although at any one time it is remarkably constant over the whole optic cortex except close to the margins. Here the spike-like prominences may tend to disappear, and the whole elevation may fail to repeat at $\frac{1}{2}$ second intervals, while still repeating in the central area. In other animals only a series of low monophasic or diphasic spikes is recorded from the periphery of the optic area. As the preparation ages, even the central areas show less repetition and less prominent spikes. This 5-per-second repetition is not always obvious in more complicated records, where the breaking up of the discharge into separate spikes may result in a nearly continuous series of spikes lasting for a second or more (fig. 5). In such cases, however, the first interval, between the shock and the main discharge, is of the usual duration and form; and as the preparation ages, the complexities tend to drop out, and the individual spikes to segregate into 5-per-second groups, which finally fuse to broad and relatively smooth waves (fig. 5, C). The course of such a transformation suggests that without an anesthetic, and in the unopened skull, the complexity of cortical activity would be such as to defeat analysis. Often, as has been described previously (Bartley and

granular layer to below cortex. *D* and *E*, surface to below cortex. The negative deflection preceding the main positive response is greater in the lower half of the cortex, but present in records from across the whole cortex.

Fig. 7. *A*, shocks to optic nerve, 1 per second, response of cortex, below maximal stimulation. Note that time between shock and main response *decreases* with repetition, although the response becomes smaller. *B*, same with stronger shocks. The time for the first response is about the same as in *A*, but the times for subsequent responses decrease less than in *A*, although the responses are larger. With still weaker shocks than in *A*, even the first response may have a shortened delay.

Fig. 8. Responses of the cortex to brief flashes of light on the retina. Three successive flashes at 1.8 second intervals. Diphasic response followed by a monophasic, similar to response to shocks on optic nerve. The interval between start of diphasic and monophasic responses is less than usual, but the occasional spontaneous rhythm appearing, shown in *C*, is also of higher frequency, being in this rabbit 7 per second. The interval between the shock and the first main response is usually somewhat less than between subsequent repetitions; in the case of electrical stimulation, typically 160 to 180 ms. shock to maximum of main response, 200 ms. between successive maxima.

Bishop, *l.c.*), one of a series of such repetitive elevations will reverse in sign, either partially, appearing as a diphasic or triphasic elevation, or nearly completely. Sometimes this may be interpreted as the result of interference by a large spontaneous wave; at other times the phenomenon is repeated at the same place in the cycle with successive stimuli. This occurrence has been too haphazard to permit of adequate investigation.

The above picture is a composite one, both with respect to time relations and form, and its details vary from case to case. For instance, the first two elements are often completely fused; but their separation in some cases with different forms serves to distinguish them as different events. One further fact is of significance in this connection. When maximal stimuli are delivered, even so infrequently as one per second, the response to the second is decidedly less in amplitude than to the first, and successive responses may decrease further. The shorter the interval between stimuli, for maximal shocks, the greater the decrease in responses after the first. When such responses are decreased, however, usually *all components* decrease, beginning with the initial discharge. That is, the cortex may follow whatever is delivered to it, other evidence indicating that the refractoriness demonstrable is at the thalamic synapses (fig. 6).

Evidence has been presented previously suggesting that the thalamus determined the rhythmic activity of the cortex, even the spontaneous activity, as well as the rhythmic alteration of excitability exhibited to sub-maximal shocks (Bishop, 1933). We now have further evidence (to be presented later) that a return circuit from cortex to thalamus is involved in conditioning the thalamus to a second stimulus from the optic nerve, and thus affecting, perhaps determining, the inherent rhythm of the whole pathway. Dusser de Barenne (1933) has emphasized the intimate relations between cortical and thalamic functioning as evidenced by strychninization. Our experiments seem to indicate that an impulse arriving at the cortex sets up an impulse that returns to the thalamus, determining its response, and through it, the response of the cortex to a second stimulus. A similar circuit is involved in spontaneous activity, which will account for the 5-per-second rhythm that both spontaneous and induced activities exhibit. That is, the time required for the impulse to make this circuit is about $\frac{1}{5}$ second; most of this time is spent in traversing the cortex; the various events described above are some indication of how the impulse is spending this time; and the return discharge to the thalamus takes place at the time of the main surface-positive wave. The natural inference is that successive repetitions of this elevation signalize successive passages of this circuit by the impulse, which, however, finally either dies away, or its elements become temporally dispersed, or both, or it merges into the spontaneous rhythm already present.

Relation of form of potential to summation of unit responses. Of the four

events which make up the first cycle of the cortical response, it has been suggested above that the first two might be fairly simple summations of diphasic potentials. The next two are effectually monophasic in the record, and might represent summations of elements more temporally dispersed. The character of such possible summation may be dealt with somewhat as follows.

The summation of any diphasic potentials, provided the two phases of the units to be summed are equal in area, should be in turn a diphasic potential, with equal areas of the two phases. The form and amplitude of the summed potential will be functions of the time relations of the unit elements, particularly of their frequency. Where the time over which summation takes place is long compared to the duration of a single element of potential, the form of the summation will be chiefly a function of the frequency rather than of the form of the unit. Such a summation may be termed a *second order* potential.

The fact that the record led from the optic radiation during the main cortical discharge has the duration of this discharge indicates that the record from the radiation, which contains only nerve fibers, is a summation of *fiber* potentials. The record from the cortex itself, since this contains the same fibers, must be in part at least a similar summation, whatever component of "slow" potentials assignable to cell bodies, after-potentials, etc., it might also involve. This cortical potential is, however, comparable to slow potentials recorded from cell masses by other workers, and its analysis becomes of considerable interest. The leads from which it is recorded are ostensibly such as to record at least some of its fibers diphasically, yet its form is typically monophasic, and its polarity such as to indicate a predominance of the second phase in the record (negativity at the second electrode along the path of the impulse, *i.e.*, the deeper of two in the cortex). The question arises to what extent the form of this potential can be explained without recourse to the assumption of long-lasting unit potentials, that is, as the summation of typical fiber potentials such as peripheral nerve trunks exhibit.

It has been noted elsewhere (Bishop, 1935) that only a slight difference in the effective areas of the two phases of the unit potentials to be summed may be required, at certain frequency distributions, to give virtually completely monophasic summation potentials. It has been suggested above that in the cortex, more second phases will be recorded from the lower strata than first phases from the upper, in an efferent discharge from the cortex, which will amount to the same thing as a difference in areas of the two phases. Not only the areas of such summated potentials, but the times of their maxima, and even their apparent starts, will depend on the frequency of response of the units of which they are the summation, whether the frequency is a matter or repetition of response in each element, or tem-

poral dispersion of responses in different elements. The frequency distribution of response may be expected to vary in the cortical discharge for numerous reasons, and certain phenomena may perhaps be explained on this basis. For instance, when successive equal shocks at one second intervals are applied to the optic nerve, the first of the series usually gives the largest response, but subsequent responses may appear *earlier* after the shock (fig. 7). It might be expected that the same factors which cause the response to be *less* should also cause it to occur *later*. On the other hand, if a first shock is in fact more effective, one of its effects should be to accelerate more rapidly the frequency of the final discharge, whether by increasing (due to facilitation between one unit and another) the frequency of repetition in each unit, or by inducing closer synchronization of the discharges in parallel units. From the fact that the cortical potential under consideration is typically monophasic, one might infer that the initial acceleration of its elementary frequency is insufficient to cause a diphasic summation. An effectively stronger (first) stimulus should then tend to render the result diphasic, and its first effect should be to bring the apparent start, and even the apparent crest of the summation, *later*. The starts of cortical potentials are difficult to measure accurately in the presence of spontaneous oscillations. The crests often behave as the above scheme suggests.

If single shocks are applied, one actually stronger than the other, the situation is similar. The stronger (below maximal) gives the greater amplitude of the main cortical response, but this response may be later than that to a weaker shock, although the initial response of the cortex is earlier, the stronger the stimulus; that is, more time is *apparently* consumed by a strong impulse traversing the cortical pathway than by a weaker one. When such shocks are delivered successively, the later responses to the strong series occur about as early as the first response to the weak series. Again an apparently less effective impulse traverses the cortex in less time than a more effective one, unless the form of the summation curve gives a deceptive picture of the time course of the cortical response.

Similar considerations might be invoked to explain the variation in the prominence of the surface-negative wave that precedes the main discharge from the cortex; but our information about this is still too unsystematic to justify elaborate speculation. Another factor, however, may be mentioned to which the foregoing arguments might be applied. If the 5-per-second repetitions of the cortex are a function of a loop pathway from cortex to thalamus and back, the repetitive discharges should involve repetitions of *all* the cortical events appearing in a first cycle. This does not appear in the records, and it may be surmised that temporal dispersion, as a cause of overlapping of the components, as well as of a change of the frequency of their units, will account for the apparent loss of all but the most prominent component. The same sort of thing should occur even in the case of the

first cycle, in the case of response to stimulation of the retina by brief flashes of light. Here the retina itself is known to give to the optic nerve not a single synchronized volley of stimuli, but a temporally dispersed repetitive discharge in the fibers activated. The result (fig. 8) is a diphasic first potential at the cortex which may be inferred to include both the afferent fiber discharge and the first cortical discharges. This is followed by a monophasic surface-positive wave comparable to the main cortical discharge. Occasionally a repetition of the latter can be detected, as in the case of direct stimulation of the optic nerve, but this is unusual, as could be expected on the basis of the arguments presented, in view of the dispersed retinal discharge of which it is the consequence.

While the cortical response to stimulation has a form that is reasonable as a summation of *fiber* potentials, it would be hazardous to infer that only fiber potentials contributed to it. It should be noted that leads from across the cortex, which is less than 3 mm. deep, compare to peripheral nerve in contact with which the recording electrodes are separated by only 2 to 3 mm. If conduction is 50 m.p.s., the interval between arrivals at the two electrodes would be 0.05 ms., or $\frac{1}{4}$ the rising phase of the action potential. This would result in an insignificant contribution of time-potential area per impulse as compared to a monophasic record, yet the responses of the cortex can be over 1 mv. in amplitude, and 100 ms. in duration. This diphasic suppression would be obviated to some unknown extent by the partially monophasic recording of part of the elements of the cortex, those arising effectively between leads. One could assume also that in the 100 ms. which the main cortical response occupies, a given element could act repetitively up to, say, 50 responses.

DISCUSSION. The foregoing experiments concern the responses of the optic cortex to stimulation of the pathway of the visual impulse. The phenomena have been discussed as if the responses were visual responses. It can only be claimed at present that they are physiological responses correlated with stimuli potentially visual. We do not know where "vision" is consummated, but the optic cortex response must be a preliminary to it. The question arises whether the cortex responds to single shocks to the optic nerve in a manner similar to its response in vision.

Two differences can be pointed out at once. First, facilitation is readily demonstrated below the level of the cortex, and the difference between the single volley sent up to this level after a single shock, and the scattered repetitive discharge of the optic nerve to retinal stimulation by light must make a difference in the organization of the response at higher levels. Presumably the same cortical elements respond in the two cases, in roughly the same sequence, but possibly with much more intimate mutual interaction in the more complicated situation. Secondly, the long "refractoriness" demonstrable after a maximal shock obviously does not apply directly in

vision, unless we compare such a shock to a blinding flash of light. To less than maximal stimulation, it has been shown (Bartley, 1936) that elements not activated by the first shock can be activated by a second shock of the same strength at any time after the first, owing to the fact that such elements become accessible to stimulation rhythmically, but usually more or less out of phase with each other. A given unit locus (in the retina) must therefore be able to employ any one of a number of elements, in projecting itself upon the cortex, and to employ them alternately, to accomplish "continuous" vision. The variations in responses we obtain may then be assigned in part to a variable number of elements receptive at a given instant, starting from a condition of "rest."

In the rabbit, the presence of the 5-per-second rhythm, corresponding presumably to the 10-per-second rhythm recorded from the occiput of many human subjects, apparently signalizes a rhythm of excitability of the optic pathway. Quite as this rhythm is broken up or decreased in the human case by light stimuli, so both the rhythmic spontaneous responses and the underlying excitability rhythm which they connote can be altered by stimulation of the optic nerve in rabbits. In the human case, the light stimulus presumably scatters the synchronism of elements whose previous summation amounts to a potential large enough to be recorded. In the rabbit, a single volley of impulses over the optic nerve resynchronizes the elements to an even more pronounced rhythm, or if the rhythm is already maximal, such a volley may be ineffective if it falls in the surface-negative inexcitable phase of the spontaneous rhythm. It would appear then that an alert or continuously receptive state is characterized by an asynchronism of the elements involved, and that in a condition of rest (lack of stimulation) such synchronization tends to be reestablished, possibly by mutual facilitation of parallel spontaneously rhythmic elements. The first part of a persistent stimulus to the eye should then be expended in reorganizing the state of "rest," and this may be a factor involved in focusing the attention on the stimulus, a process that notoriously requires time.

The spontaneous rhythm exhibited, as well as part of the response to stimulation, may then be assigned to coördinating circuits whose function is to facilitate the visual pathway, but whose activity alone does not involve vision. Presumably another part of the response to stimulation, whose magnitude is conditioned by these coördinating circuits, is involved in transmitting the visual impulse itself. In accordance with this, a response to stimulation can always be obtained which is much greater in amplitude than any spontaneous activity. The mode of action indicated here involves something like the reverberating circuits for which Lorente de N6 (1934b) has found histological evidence in the cortex.

Certain differences between responses to light on the retina and to shocks across the optic nerve are therefore assignable primarily to differences in

the organization of the impulse at the level of the thalamus and above, and aside from this, the cortex appears to respond to each in accordance with the same essential characteristics. The record is simpler in appearance and larger in amplitude when the elements responding are as much as possible summated, and the record disappears when, under continuous stimulation by light, the responses of individual elements are completely asynchronous, only the "on" and "off" effects appearing in the cortical record. Single shocks to the optic nerve then represent the opposite extreme to continuous stimulation of the retina by light, so far as the cortical response is concerned, and enough intermediate stages are becoming known, through repetitive shocks on the one hand or repetitive short flashes of light on the other, to effectively bridge the gap between them, and allow each to be interpreted in terms of the other. It thus appears that the more complicated the stimulus, the more simple is the *record* of the response. It is reasonable to infer that the visual act is not accomplished without each chain of elements comprising a unit pathway from retina to brain being occupied by the activity of which the cortical record to a single shock is an index, even though an incomplete one. This record differs from the record of a response to retinal stimulation chiefly in that the latter is the more confused picture, that is, it is the more complex in the temporal relations of its elements.

SUMMARY

A single maximal stimulus applied to the fibers of the optic nerve results in a response of the optic cortex of which four components can be differentiated. The first two are diphasic potentials, often fused to one, of which the first phases involve positivity of the surface of the cortex. The third is a slow surface-negative deflection, the fourth a slow surface-positive deflection, the latter often having superposed upon it a series of monophasic or diphasic spikes. When smooth, it perhaps consists of the smooth summation of such spikes temporally dispersed. The fourth component at least is typically repeated at about $\frac{1}{5}$ second intervals during one second or less.

These components, any two of which may be confluent in a given record, are differentiated by their differences of form when separate, by their differential changes under manipulation, and by the fact that they are recorded differently when led from different levels in the vertical stratification of the cortex.

Considerations of the relation of the form of unit potentials (potentials of unit neural elements) to the form of the summated response, indicate that the form of the summated wave will be a function of the degree of diphasicity of the unit elements as led off, of the spatial relations of the units to the electrodes, and of the acceleration of the frequency of unit

responses, aside from the mere numbers of such units activated by a given stimulus.

REFERENCES

- BISHOP, G. H. This Journal **103**: 213, 1933.
Arch. Ophth. **14**: 992, 1935.
BARTLEY, S. H. J. Cell and Comp. Physiol. **8**: 41, 1936.
BARTLEY, S. H. AND G. H. BISHOP. This Journal **103**: 159, 1933a.
This Journal **103**: 173, 1933b.
CAJAL, S. RAMON Y. J. Psychol. and Neurol. **29**: 161, 1922.
DUSSEY DE BARENNE, J. G. Arch. Neurol. and Psychiat. **30**: 1, 1933.
POLIAK, S. Univ. Colorado Publ. in Anat. **2**: 369, 1932.
LORENTE DE NÓ, R. J. Psychol. and Neurol. **45**: 381, 1934a.
J. Psychol. and Neurol. **46**: 113, 1934b.
ROSE, M. J. Psychol. and Neurol. **43**: 353, 1931.

ABSORPTION OF SODIUM CHLORIDE FROM THE SMALL INTESTINE AT VARIOUS DEGREES OF ANOXEMIA¹

EDWARD J. VAN LIERE AND CLARK K. SLEETH

*From the Department of Physiology, West Virginia University, Morgantown,
West Virginia*

Received for publication June 26, 1936

In a recent paper the effect of anoxemia on absorption of water from the small intestine was reported by Van Liere, David and Lough (1936). In this paper it was shown that absorption of water takes place much more readily at rather pronounced low oxygen tensions than at normal atmospheric pressure. In view of these findings it was thought worth while to study the absorption of an electrolyte from the gut under anoxic conditions.

Hamburger (1896) found that absorption of salt solutions from the intestines of dogs which had been dead from one to twenty-four hours proceeded in the same manner as living dogs. Magee and Macleod (1929) reported that the walls of segments of gut which had been devitalized became more permeable to solutions and electrolytes than normal gut. One might deduce from these experiments that oxygen is not absolutely necessary for absorption of electrolytes from the intestine.

METHODS. With slight variations the same methods were used as those reported in the paper already mentioned. Barbitalized dogs and cats which had been starved twenty-four hours previous to the experiment were used. Two animals were chosen which were as near the same weight and age as it was possible to obtain them. In a number of experiments litter mates were used. One animal was used as a control and the other subjected to anoxemia.

A midline incision was made in the abdominal wall and the small intestines exposed. Practically the entire small intestine with the exception of the duodenum was used for a loop. The loops of the two animals were made of equal length by actual measurement. They were washed out with 5 per cent glucose solution; care was exercised to recover all the fluid which had been used for washing.

A measured amount of 0.9 per cent sodium chloride solution was placed in each loop. Distention was avoided. The intestines were replaced in

¹ The expenses of this investigation were defrayed by a grant from the Committee on Scientific Investigation of the American Medical Association.

the abdominal cavity and the wound closed with sutures. The animal to be subjected to anoxemia was placed in a steel respiratory chamber pre-

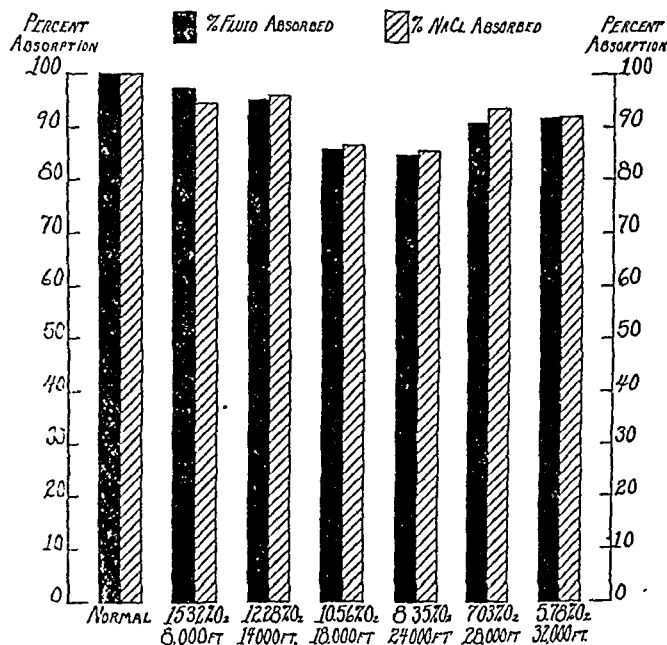


Fig. 1

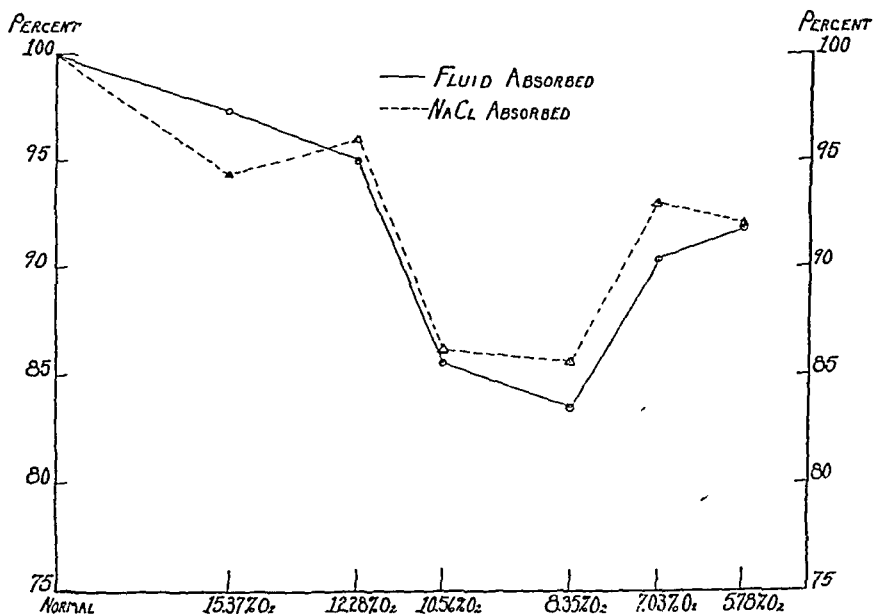


Fig. 2

viously described (Van Liere, 1927). The one which was to serve as a control was kept at atmospheric pressure. The salt solution was left in the intestinal loops for 40 minutes. The loops were then removed from the

body of the animal, slit open and the contents carefully measured. The actual amount of sodium chloride in the recovered fluid was determined by Van Slyke's modification of the Vollhard method.

The effect of the following percentages of oxygen on the absorption of salt solution from the small intestine was determined: 15.32, 12.28, 10.56, 8.35, 7.03 and 5.78.

RESULTS. The accompanying graph and chart show the results obtained. It will be seen that anoxemia curtailed the amount of fluid and sodium chloride absorption. Anoxemia was most effective in decreasing the amount of absorption at oxygen percentages of 10.56 and 8.35. Lower percentages of oxygen (7.03 and 5.78) did not decrease the amount of absorption as much as did the oxygen percentages formerly mentioned. The fluid absorption and actual sodium chloride absorption practically ran parallel.

DISCUSSION. The results reported here are quite different from those reported in a previous paper in which the effect of anoxemia on water absorption from the small intestine was studied. In the latter study it was reported that 15.37 per cent oxygen had no appreciable effect on the absorption of water from the small intestine, but that there was a decrease in the amount of absorption at 12.28 per cent oxygen. At higher degrees of anoxemia, however, absorption took place more readily from the small intestine in the anoxemic animals than in the controls.

The absorption of 0.9 per cent sodium chloride solution was diminished at all degrees of anoxemia. A significant reduction in the amount of absorption took place even at 15.32 per cent oxygen. This is a very mild degree of anoxemia and corresponds to an altitude of about 8000 feet. Many people live at such altitudes.

It is difficult to explain the fact that severe degrees of anoxemia are less effective in reducing the amount of absorption of 0.9 per cent sodium chloride from the small intestine than more moderate grades of anoxemia. It must be stated here that 126 animals were used in these experiments and with such a large series we feel that our results are fairly accurate. It may be that extremely low oxygen tensions actually injure the cells in the intestine and this may make them more permeable to the passage of the sodium chloride molecule. The lowest oxygen percentage used was 5.78 as it was found that the majority of barbitalized animals could not withstand lower oxygens tensions than this for the time necessary to perform the experiments.

It is hardly proper to compare the results reported in this paper with those obtained by Hamburger or those of Magee and Macleod. These authors worked with dead membranes and it is quite likely that dead membranes have a different permeability than do living ones.

The question of the reaction of the blood during anoxemia, and its effect

on absorption of water has been discussed at some length in a preceding paper (Van Liere, David and Lough). It is, of course, well known that the more severe the degree of anoxemia the lower the pH of the blood becomes. As the graph and chart show, however, there is no direct ratio between the amount of absorption of 0.9 per cent sodium chloride solution and the degree of anoxemia. The reaction of the blood, therefore, cannot be used to explain the reason for severe degrees of anoxemia being less effective in reducing the amount of absorption than more moderate degrees.

SUMMARY AND CONCLUSIONS

In studying the effect of various degrees of anoxemia on the absorption of 0.9 per cent sodium chloride solution from the small intestine of barbitalized dogs and cats it was found that low oxygen tensions decreased the amount of absorption. Even a mild degree of anoxemia (15.32 per cent oxygen) had a significant effect on absorption. The fluid absorption and the actual sodium chloride absorption practically ran parallel.

The greatest decrease of absorption of 0.9 per cent sodium chloride solution from the small intestine occurred at 10.56 and 8.35 per cent of oxygen. At more severe degrees of anoxemia (7.03 and 5.78 per cent of oxygen) absorption was not decreased as much as in the two ranges previously mentioned. It is suggested that extremely low oxygen tensions actually injure the cells of the intestinal membrane and that permeability is increased in this manner.

The conclusion which may be drawn from this work is that oxygen aids in the absorption of 0.9 per cent sodium chloride from the small intestine of the mammal.

REFERENCES

- HAMBURGER, H. J. *Arch. f. Anat. u. Physiol.* 428, 1896.
MAGEE, H. E. AND J. J. R. MACLEOD. *This Journal* 90: 442, 1929.
VAN LIERE, E. J., N. A. DAVID AND D. H. LOUGH. *This Journal* 115: 239, 1936.
VAN LIERE, E. J. *This Journal* 82: 727, 1927.

TRANSPLANTATION OF SINO-ATRIUM TO CONUS IN THE EMBRYONIC HEART IN VITRO

GEORGE H. PAFF

From the Department of Anatomy, Long Island College of Medicine, Brooklyn, N. Y.

Received for publication June 26, 1936

The production of an ectopic pace-maker by transplantation of the sino-auricular node in adult hearts (7) leads to the question as to what would occur following a similar procedure in tubular embryonic hearts. Concerning the pace-maker in embryonic hearts no circumscribed mass of tissue comparable to a node can be distinguished. It is well established, however, that there exists a gradient of rhythmicity inclined from the venous to the arterial end. Furthermore the separation by section or ligation of sino-atrium from ventricle results in a difference in rate of beat of the two moieties, the venous portion contracting at a distinctly faster rate (2, 6, 3, 4, 5). It has also been shown by Paff (5) that the sino-atrial region dominates the remainder of the heart. Complete transection with accompanying functional disunion as manifested by a marked decrease in the rate of the ventricle is, under proper conditions, followed by reestablishment of functional union. This is demonstrated by a marked "stepping up" of activity in the ventricle as new growth bridges the region of transection. With reestablishment of a conductile pathway the beat in the two portions becomes synchronous again.

In the present group of experiments the author has transplanted the sino-atrium from the ventricle to the conus (cf. fig. 1) to determine whether or not it is possible for the pace-maker to dominate the ventriculo-conal region from this reversed location.

METHOD. Hearts were dissected from 55 hour chick embryos and washed in Tyrode solution. The sino-atrium was cut free from the ventricle and placed in apposition to the opposite end or the conus arteriosus (cf. fig. 1). The preparation was then very gently flooded with chicken blood plasma and mounted over a depression slide. Embryonic extract was not used.¹

¹ In conducting these experiments it was felt that results would be more conclusive if each heart were preserved as a heart in the cultures. Too often in tissue cultivation the investigator is interested primarily in rapid growth. The companion to rapid growth is migration of cells. In preliminary experiments fresh embryonic extract and blood plasma were used. Results were discouraging. Growth was rapid and migration was extensive. In some cases the hearts lost their identity in twenty-four hours. Because of this, embryonic extract with its growth promoting substances was not used. Blood plasma supported sufficient growth and throughout the experimental period the hearts were identifiable as hearts rather than just pulsating masses of protoplasm.

Observations were made with the preparation in a warm chamber at 38°C. In taking rates of contraction the author's counts were confirmed by other observers. Eight successful experiments were obtained, and of this number data are presented below for four.

OBSERVATIONS. Following removal and sectioning of the hearts, the ventriculo-conal region beats at a distinctly lower rate than does the sino-atrium. Placement of the sino-atrium to the opposite end of the heart results at first in no alteration in this relationship. In a variable number of hours, however, striking changes become apparent. The two portions of the heart begin to grow together and then the behavior of the ventricle changes. The first expression of this is usually seen as a struggle to maintain its own rhythm against the opposing one of the sino-atrium. As growth in uniting tissue progresses, it is common to observe an apparent two to one rhythm between sino-atrial and ventriculo-conal regions. Within a few hours this relationship gives way to periods of synchronous

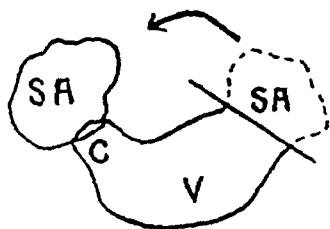


Fig. 1



Fig. 2

Fig. 1. Heart 13 immediately after removal from embryo. Change of position of sino-atrium, SA from ventricle, V, to conus, C, indicated by arrow. Outline tracing x 28.

Fig. 2. Heart 13 after 27 hours in vitro. Tracing of heart at time when rhythm was synchronous superimposed on photograph taken after final observations. Plane of section indicated by solid line. Hematoxylin stain x 28.

activity during which the rate of the ventricle is increased to correspond to that of the sino-atrium. These periods of synchronicity alternate with ones during which the ventricle beats at a slower rate than the sino-atrium, sometimes in one to two relationship, sometimes quite independently. Finally a regular synchronous rhythm is established and persists.

After the establishment of a synchronous rhythm a significant change occurs when the hearts are transected in the region of new union between sino-atrium and conus (cf. fig. 2). Sectioning terminates the unified activity. Released from the sino-atrium, the ventriculo-conal region takes up its own rhythm, the rate falling distinctly below that of the sino-atrium. In the latter the rate is practically unchanged.

Fundamentally the behavior in each heart was the same. However, in certain particulars sufficient variation occurred as to make it advisable to present specific data for at least four of the eight successful experiments.

Heart 1. Hours after planting

1 HOUR	23 HOURS	25 HOURS	29 HOURS (CUT)
SA—#186 VC— 69	SA—149 VC—126	SA and VC—142 (Synchronous)	SA—146 VC— 68

SA—Sino-atrium; VC—ventricle conus; #—number of beats per minute.

In heart 1, after 23 hours in vitro, periods of synchronicity alternated with periods of two to one activity. At 25 hours the beat was synchronous. This was difficult to decide at a temperature of 38°C. because the heart was beating so rapidly. By dropping the temperature several degrees the activity decreased sufficiently to permit of an unquestionable conclusion that the beat was synchronous. After sectioning at the place of union the rate of beat in the sino-atrium was changed but little (142 to 146 per minute). In the ventricle the decrease was marked (142 to 68 per minute).

Heart 13. Hours after planting

1 HOUR	19 HOURS	23 HOURS	27 HOURS (CUT)
SA—164 VC—120	SA and VC—94 (Synchronous)	SA and VC—84 (Synchronous)	SA—89 VC—62

In heart 13, when observed 17 hours after planting, a struggle seemed to be taking place between sino-atrium and ventriculo-conal region. For brief periods of variable duration they were synchronous. Within 2 hours (19 hrs.) a regular synchronous rhythm was established. Here again after cutting at 27 hours, the sino-atrium remained quite constant (84 to 89) but the ventricle and conus changed (84 to 62).

Heart 9. Hours after planting

1 HOUR	18 HOURS	21 HOURS	25 HOURS (CUT)
SA—180 VC—134	SA and VC—34 (Synchronous)	SA and VC—94 (Synchronous)	SA—96 VC—72

At 18 hours heart 9 was beating synchronous but the heart showed periods of activity alternating with periods of rest. This is a phenomenon which occurs frequently with prolonged cultivation and persists until transplantation to fresh medium. In this case, under uniform conditions, it gradually disappeared and three hours later the heart was again beating regularly. Note the decrease in rate of ventricle following sectioning at 25 hours.

Heart 8. Hours after planting

1 HOUR	20 HOURS	24 HOURS	28 HOURS (CUT)
SA—144 VC—122	SA and VC—96 (Synchronous)	SA and VC—115 (Synchronous)	SA—113 VC— 70

This preparation showed an unusually vigorous beat. Following synchronization, ventricular systole produced a decrease in diastolic diameter of fully 25 per cent. After cutting the region of union at 28 hours, the ventriculo-conal region was far less vigorous and its rate dropped from 115 to 70 per minute.

DISCUSSION. Following the transplantation of the pace-maker and the production of a unified rhythm, the control of the sino-atrium over the ventriculo-conal region is proved by the fact that when the two are separated by transection the ventricle and conus again drop distinctly in rate of beat. This difference per minute in the four preparations presented was 78, 27, 24 and 43. In contrast to this, the sino-atrium maintains practically the same rate of beat as observed in the heart before transection (at the region of union). The question arises as to whether the ventricle and conus have been activated in the reverse direction. That this is so is proved by the fact that if, after a unified rhythm is established, the heart is made quiescent by cooling and then reactivated by warming, the sino-atrium beats first and is followed a split second later by the ventricle. As the rate increases each beat originates in the sino-atrium before the previous one is completed in the ventricle. Assuming the existence of a refractory period comparable in duration to that of the adult heart, the effective impulse is traversing the conus while the previous impulse is producing the wave-like systole of the ventricle.

It may also be significant that small masses of corpuscles within the lumen of the hearts are given an initial thrust toward the end of the ventricle opposite to the new sino-atrial location. In reference to this point Bremer (1) observed reversal of the beat in the heart of an embryo in situ, in which case blood was actually being pumped out of the veins. In his experiment the conus gained the ascendancy over the sino-atrium. Such a condition is exceptional but it leaves no doubt that reversal of the normal gradient in ventricle and conus is possible.

In establishing this new functional relationship between sino-atrium and ventricle certain conditions must be met. If union is to occur within twenty-four hours the cut surfaces of each moiety must be brought close together. Attempts to graft the sino-atrium on the intact lateral border of the conus met with no success in forty-eight hours of cultivation. Each portion of the heart contracted independently.

As a corollary to the above condition, myocardium must knit to myocardium. The embryonic muscle alone will furnish the anatomical basis for a functional pathway. In over a hundred experiments synchronization was never observed in sectioned hearts as the result of fusion of fibroblasts from the cut ends of the two parts.

CONCLUSIONS

1. The sino-atrium when transplanted to the conal end of the heart will, under proper conditions, dominate the ventriculo-conal region.
2. In the ventriculo-conal region activity is reversed.
3. The anatomical requirement is union of myocardium of sino-atrium to myocardium of the conus.

REFERENCES

- (1) BREMER, J. L. Anat. Rec. **51**: 279, 1932.
- (2) FANO, G. AND F. BADANO. Arch. Ital. di Biol. **13**: 410, 1890.
- (3) JOHNSTONE, P. N. Bull. John Hopkins Hosp. **35**: 87, 1924.
- (4) LEWIS, W. H. Bull. John Hopkins Hosp. **35**: 255, 1924.
- (5) PAFF, G. H. Anat. Rec. **63**: 203, 1935.
- (6) PICKERING, J. W. J. Physiol. **14**: 391, 1893.
- (7) RIJLANT, P. Arch. Intern. de Physiol. **28**: 225, 1927.

INCREASED WATER EXCHANGE FOLLOWING ECK FISTULA IN DOGS

LATHAN A. CRANDALL, JR. AND GEORGE M. ROBERTS

*From the Department of Physiology and Pharmacology, Northwestern University
Medical School, Chicago*

Received for publication June 27, 1936

Since Molitor and Pick (1) showed that water by mouth in dogs with Eck fistulae produces a greater blood dilution and a more prompt diuresis than occurs in normal animals, there have been numerous observations indicating that the liver plays a part in regulating the water content of the blood (2, 3, 4, 5, 6, 7, 8). It occurred to us that if a normal function of the liver is to retain a large proportion of ingested water and gradually release it to the blood, disturbance of this function, leading to more prompt elimination of water, should result in an earlier appearance of thirst and an increased intake and output over long periods of time. We have therefore studied the voluntary fluid intake and urinary excretion in dogs before and after the establishment of Eck fistulae.

METHODS. Normal dogs, kept in cages from which urine could be collected, were allowed access to water *ad lib.*, a 2 or 5 gallon crock being present in the cage at all times. The diet consisted of a mush made of bread and corn meal boiled with bones. The voluntary water intake and the urinary output were measured daily over a control period of at least 2 weeks. An Eck fistula was then established under Nembutal anesthesia, the animal allowed to recover from the immediate effects of the operation for 48 hours and observations on intake and output continued. As a further control, a dummy operation which consisted simply of stripping the portal vein and the vena cava as in preparation for an Eck fistula was performed on 2 dogs. This procedure was designed to produce as great an interference with the nerve supply of the liver as may be present in the Eck fistula animals. To further eliminate the possibility that destruction of a part of the nerve supply to the liver or other organs was responsible for the observed changes, intake and output were followed in 2 dogs before and after section of both splanchnic nerves. The influence of other procedures that damage the liver was observed in 2 dogs with ligation of the common bile duct and in 2 that were each given a one hour period of deep chloroform anesthesia.

Since not all dogs responded to the establishment of an Eck fistula with a markedly increased water exchange, it was of interest to correlate the

increase in intake and output with the degree of blood dilution that occurred after giving water by mouth. For this purpose we gave 20 cc. of water per kilo by stomach tube to 6 Eck fistula dogs on which intake and output were measured, and followed the change in the plasma chlorides at 20 minute intervals for 80 minutes. The maximum blood dilution taking place after this amount of water by mouth was determined on 10 normal animals, as was the average normal intake and output for the same season over a two week period. It is of incidental interest that a maximal dilution of the chlorides was produced by 20 cc. of water per kilo; 30 or 40 cc. per kilo was not always retained and in any case caused no greater decrease in the plasma chloride.

RESULTS. In table 1 are presented the daily voluntary water consumption of 8 dogs before and after the establishment of the Eck fistulae. Urinary output is not given, since it ran parallel to the water consumption,

TABLE 1

Effect of Eck fistula on the average daily water intake (figures represent averages of daily intakes for periods of one week)

DOG	PRE-OPERATIVE, WEEKS				POST-OPERATIVE, WEEKS							
	4	3	2	1	1	2	3	4	5	6	7	8
E-1		310	350	330	2,900	2,730	2,050	1,660	1,630	1,480		
E-2		371	230	270	450	290	245	420	370	230		
E-3			385	450	1,630	1,800	1,630	1,340	1,050	870	910	730
E-4	205	250	185	190	1,500	760	550	350	380	350	190	500
E-5			270	320	1,870	1,620	1,510	1,230	1,420	1,820	1,920	1,370
E-9			310	290	891	373	285	295				
E-10		180	184	166	671	340	285	295				
E-11			370	400	900	750	710	710	800			

which it commonly exceeded by 100 to 200 cc. The water taken in the food was not measured. The specific gravity of the urine was determined daily on a number of the animals; it was roughly in inverse proportion to the volume of urine excreted. The data on intake and output following the dummy operation, ligation of the common bile duct, splanchnic section, and administration of chloroform, are given in table 2. The only one of these procedures that appeared to affect water exchange was chloroform poisoning, which produced an increase comparable to that following an Eck fistula. Table 3 gives the correlation between voluntary intake and degree of chloride dilution following water by mouth in 6 Eck fistula dogs; data on chloride dilution and voluntary intake in 10 normal dogs are submitted for comparison. The correlation is surprisingly good. None of the Eck fistula animals that were at hand at the time the studies were made exhibited an average daily intake within the normal range, but the two

TABLE 2

Effect of splanchnicotomy, common bile duct ligation, stripping of veins, and chloroform on average daily intake

(S—splanchnicotomy, B—duct ligation, D—veins stripped, C—chloroform)

DOG	PRE-OPERATIVE, WEEKS			POST-OPERATIVE, WEEKS			
	3	2	1	1	2	3	4
S1		230	270	330	270	265	
S2		180	165	140	160	135	
B1		90	75	80	60	110	
B2		310	280	350	270	310	
D1	210	300	250	175	180	185	350
D2	425	400	370	250	260	320	240
C1		340	400	1,070	900	920	
C2		90	110	200	180	170	

TABLE 3

Correlation between voluntary daily intake and blood chloride dilution after water by mouth

- A. Daily water intake per kilo in 10 normal dogs; average of determinations over a 2 week period
- Average, 23 cc.
Maximum, 32 cc.
Minimum, 20 cc.
- B. Per cent decrease of plasma chloride in 10 normal dogs after 20 cc. of water per kilo by mouth
- Average, 2.8
Maximum, 5.0
Minimum, 1.2
- C. Voluntary intake compared with per cent fall plasma chloride in Eck fistula dogs

DOG	PER CENT FALL CHLORIDE AFTER 20 CC. WATER PER KILO BY MOUTH	DAILY WATER INTAKE PER KILO; AVERAGE OF TWO WEEK PERIOD
E-6	5.0 4.2	55
E-7	9.0 7.8	73
E-8	7.0	46
E-9	2.7 2.5	37
E-10	1.5 3.0	34
E-11	7.5 6.3	57

(E-9 and E-10) that exceeded the normal intake by only a small margin showed a chloride dilution well within the limits found in normal dogs. In the remaining four, the increase in output is roughly proportional to the degree of chloride dilution. Maximum dilution was usually present at the 20 or 40 minute interval.

It should be noted that the subcutaneous injection of pituitrin had no effect on the increased intake and output of the Eck fistula dogs.

DISCUSSION. The obvious explanation for the greatly increased water exchange that follows hepatic damage is based upon the same reasoning that induced us to perform these experiments. The greater blood dilution occurring after water by mouth in animals and patients with liver injury has been adequately confirmed (1, 2, 3, 4). According to Molitor and Pick (1) there is also a more prompt diuresis and a greater total excretion. Although little is known concerning the mechanism of thirst, it can be assumed that the prompt elimination of fluid would result in a more prompt reappearance of the desire for water.

Because the surgical procedures involved in making an Eck fistula probably produce some interference with the nerve supply of the liver, the control experiments of stripping the vena cava and portal vein (dummy Eck fistula) and sectioning both splanchnics were performed. Since neither of these procedures influenced the water exchange, while chloroform poisoning, involving no operative interference, did increase intake and output, we may assume that the change is produced by a direct suppression of the function of the parenchymatous tissue. While a high degree of liver injury also eventually occurs after ligation of the common bile duct, this procedure introduces digestive disturbances and other complicating factors. The pathological changes in the liver following duct ligation are known to be progressive, but our animals were studied for three weeks only because of the cachexia that developed.

The fact that the specific gravity of the urine fell precipitately (values of 1.002-1.004 were common) after the Eck fistula operation indicates that the greater water exchange does not result from the liberation and excretion of stored salts. Further, the serum osmotic pressure of our animals, as determined by the freezing point method, remained within normal limits.

It is well known that there is a considerable variation in Eck fistula dogs, some showing little observable change, others exhibiting symptoms promptly and dying within a few months, while the majority run an intermediate course. Therefore it is not surprising that the effect on water exchange was also variable. We noted that the animals which drank and excreted the largest amounts of water post-operatively exhibited the characteristic symptoms, while those in which the water exchange was little affected showed relatively little change from the normal in other respects.

The duration of the effect on water exchange was not carefully studied. In dog E-1 the intake and output were still approximately double the control level in the ninth post-operative month, although greatly decreased from the 800 per cent rise that occurred immediately after the operation. Dog E-7 was used primarily for other studies, but 10 months after operation it was noted that his voluntary intake per kilo was approximately 3 times the average of our normal series and more than twice as great as the maximum normal.

The observed correlation between plasma chloride dilution after water by mouth and voluntary 24 hour intake was to be expected if the increased water exchange is secondary to a reduction in the ability of the liver to store water. Baldes and Smirk (9) have presented evidence indicating that urine formation is largely controlled by changes in the total osmotic pressure of the blood. The greater blood dilution and more prompt and complete urinary excretion of ingested water in animals with liver damage supports this concept. It also seems that interference with the water storage function of the liver, resulting in a more prompt elimination of ingested water, leads to an earlier reappearance of thirst. Repetition of the cycle brings about an increase in voluntary water consumption and urinary output over long periods of time.

SUMMARY

Establishment of Eck fistulae in dogs leads, in the majority of instances, to a lasting increase in voluntary water intake and urinary output. Chloroform poisoning acts similarly, while a dummy Eck fistula operation, splanchnic section, or bile duct ligation is without effect. The increased water exchange is correlated with a greater dilution of the plasma chlorides after oral administration of water. The effect appears to be secondary to interference with the water storage function of the liver, with a consequent prompt elimination of water and reappearance of thirst.

REFERENCES

- (1) MOLITOR, H. AND E. P. PICK. *Arch. f. exper. Path. u. Pharmacol.* **97**: 317, 1923.
- (2) KISS, J. *Deutsch. Arch. f. klin. Med.* **157**: 202, 1927.
- (3) LANDAU, N. AND L. VON PAP. *Klin. Wehnschr.* **2**: 1399, 1923.
- (4) ADLER, A. *Klin. Wehnschr.* **2**: 1980, 1923.
- (5) LAMSON, P. D. *J. Pharmacol. Exper. Therap.* **16**: 125, 1920.
- (6) ADOLPH, E. F., M. J. GERBASI AND M. J. LEPORE. *This Journal* **107**: 647, 1934.
- (7) MARSHALL, H. T., B. F. AYDELOTTE AND H. G. BARBOUR. *This Journal* **98**: 615, 1931.
- (8) BARBOUR, H. G. AND B. F. AYDELOTTE. *This Journal* **104**: 127, 1933.
- (9) BALDES, E. J. AND F. H. SMIRK. *J. Physiol.* **82**: 62, 1934.

THE GLUCOSE UTILIZATION OF PHLORIDZINISED DOGS AFTER HEPATECTOMY

D. R. DRURY, H. C. BERGMAN AND PAUL O. GREELEY

*From the Department of Physiology, School of Medicine, University of
Southern California*

Received for publication June 29, 1936

The rate of glucose utilization by the tissues of the phloridzinised dog has an important bearing on the theory of the intermediary metabolism of foodstuffs. After three days of fasting and phloridzin administration the readily-available glycogen of the body has been removed, and glucose that is excreted after this comes from non-carbohydrate sources. From the nitrogen excreted in the urine, the amount of protein catabolised may be determined, and if it be assumed that no sugar can arise from fat the urinary glucose must come from protein catabolised. The dextrose: nitrogen ratio of the urine of such animals is 3.65:1 and since each gram of urinary nitrogen is supposed to come from 6.25 grams of protein, it is claimed that 6.25 grams of protein can give rise to 3.65 grams sugar. However in such calculations no consideration is given to the glucose which may be oxidized in the tissues, presumably it being assumed that none has such a fate. Wierzuchowski (1927) and Deuel, Wilson, and Milhorat (1927) have shown that the phloridzinised dog is capable of oxidizing administered glucose. The question to be answered is, are the tissues of the phloridzinised animal actually utilizing glucose when none has been administered, and the only source of it is that formed in the body from other foodstuffs. All evidence today points to the liver as the site of this transformation. The amount of such new formed sugar will equal the sugar excreted plus the glucose oxidised by the tissues. If the liver be removed the source of glucose within the body is eliminated and in order to maintain the blood sugar level constant, glucose must be supplied to the animal at a rate at least equal to the rate of excretion by the kidneys. If more than this must be given to maintain the level then we may presume that the tissues are utilizing the substance.

METHOD. Stated simply the method consists of producing the phloridzinised state (three or more days of fasting and phloridzin), next removing the liver and then maintaining, with intravenous glucose, the blood sugar level that was found immediately before operation. In some experiments the kidneys were removed and in the others the bladder was emptied and

washed after operation and urine collected thereafter, to determine the sugar excreted. In the later experiments blood lactic acid and muscle glycogen determinations were made on samples taken at the beginning and end of the experimental period, in order to check up on every possible source or loss of carbohydrate.

Phloridzin was administered daily, subcutaneously, the dosage being one gram suspended in olive oil. Hepatectomy was performed by the method of Markowitz and Soskin (1927) as modified by Drury (1929). Blood sugar determinations were carried out by the method of Folin and Wu. For glucose estimation on the urine the Shaffer-Hartmann method was used. For muscle glycogen determinations whole muscles were used, the flexor carpi radialis of the fore leg is suitable for this purpose: it weighs 2 to 3 grams, and can be removed, using local anesthesia, by cutting its tendons at either end. The muscle of one leg is taken at the beginning of the period and this is compared with the corresponding muscle of the other leg which is taken at the end. Pflüger's procedure was followed, with Shaffer-Hartmann determination of glucose after hydrolysis. Lactic acid determinations on blood and urine were carried out by the method of Friedman, Cotonio and Shaffer as modified by Wendell (1933).

The actual routine of the experiments was as follows: The dog, after the phloridzin period, was brought to the laboratory and a blood sample was taken for sugar estimation. Hepatectomy was then done under ether anesthesia. As soon as the liver vessels were tied, constant intravenous injection of a 5 or 10 per cent glucose solution was started, this being delivered from a burette, through a dropping funnel, to a needle kept in place in one of the hind leg veins. Blood sugar estimations were done every half hour or less, and the injection rate was adjusted accordingly, the attempt being made to keep the blood sugar level the same as that found immediately before the operation. As soon as the operation was completed, the urinary bladder was washed out, a blood sample was taken for lactic acid, and a muscle for glycogen, determinations. The animal was then carried for several hours, and the experiment was completed by collecting all the urine secreted since the first washing, and by taking samples for determination of muscle glycogen and blood lactic acid.

RESULTS. The following is the protocol of a typical experiment in which the kidneys were not removed.

Dog 1. 4-15-36 to 4-19-36. Fasted and given 1 gram phloridzin in olive oil subcutaneously every day (5 days).

4-20-36 9:00 a.m. Weight 12.7 kgm. Blood sugar 50.

9:05 Ether started for hepatectomy.

9:55 Portal vessels tied. Intravenous injection of 10 per cent glucose started, burette reading at 0.0.

10:12 Blood sugar 72. Burette reading 3.4.

10:53	Blood sugar 78. Burette reading 10.1.
10:55	Operation ended.
11:20	Muscle taken from right fore leg. Glycogen concentration = 242 mgm. per cent. Blood lactic acid = 121 mgm. per cent.
11:27	Blood sugar 56. Burette reading 13.0.
11:44	Bladder washed and urine collection started. Burette reading 14.5.
12:10 p.m.	Blood sugar 57. Burette reading 16.9.
12:44	Blood sugar 52. Burette reading 20.3.
1:12	Blood sugar 47. Burette reading 22.8.
1:43	Blood sugar 42. Burette reading 26.4.
2:15	Blood sugar 45. Burette reading 31.6.
2:45	Blood sugar 57. Burette reading 37.8.
3:14	Blood sugar 60. Burette reading 42.5.
3:53	Blood sugar 51. Burette reading 47.0.
3:50	Blood lactic acid = 216 mgm. per cent.
3:57	Urine collection stopped. Bladder washed.
4:20	Muscle removed from left fore leg. Muscle glycogen = 189 mgm. per cent.

The urine passed between 11:44 a.m. and 3:57 p.m. had a volume of 60 cc. and contained 1,488 mgm. glucose and 258 mgm. lactic acid. During this period 3,250 mgm. were injected. The difference 1,762 mgm. represents glucose used by the animal. (The drop in blood sugar was slight so that the correction for this would be small.)

The following is the protocol of an experiment in which the kidneys were removed at the same time as the liver.

Dog 2. 12-16-35 to 12-19-35. Phloridzin and fasting as in previous experiment (4 days).

12-20-35	9:20 a.m.	Weight 6.35 kilos. Blood sugar 46 mgm. per cent.
	9:25	Ether. Operation—hepatectomy and double nephrectomy.
	9:53	Portal vessels tied and constant intravenous injection of 5 per cent glucose started. Burette reading 0.0.
	10:11	Blood sugar 56. Burette reading 4.0.
	10:35	Operation finished.
	10:43	Blood sugar 59. Burette reading 7.2.
	11:11	Muscle taken glycogen concentration = 187 mgm. per cent.
	11:15	Blood sugar 55. Burette reading 10.2.
	11:18	Blood lactic acid = 149 mgm. per cent.
	11:45	Blood sugar 51. Burette reading 12.8.
	12:15	Blood sugar 43. Burette reading 14.0.
	12:45	Blood sugar 42. Burette reading 18.0.
	1:15	Blood sugar 44. Burette reading 22.0.
	1:45	Blood sugar 41. Burette reading 27.2.
	2:17	Blood sugar 45. Burette reading 35.0.
	2:52	Blood sugar 38. Burette reading 38.0.
	2:50	Blood lactic acid. 189 mgm. per cent.
	2:52	Muscle taken glycogen concentration = 90 mgm. per cent.

Between 11:15 a.m. and 2:52 p.m. the animal received 1,410 mgm. glucose. The

blood sugar level dropped during this period so that the utilization during this period was somewhat higher than this.

The results of the other experiments will be given briefly.

Dog 3. Three days phloridzin and fasting.

Hepatectomy only. Weight at operation 17 kilos.

In 4 hours and 28 minutes received 8,950 mgm. glucose intravenously.

In this period excreted 4,750 mgm. glucose. Blood sugar before operation 60. At beginning of experimental period 63; at end, 54. Muscle glycogens and blood lactic acids not done.

Dog 4. Three days phloridzin and fasting.

Hepatectomy and nephrectomy. Weight at operation 5.9 kilos.

In 5 hours and 58 minutes received 5,138 mgm. glucose intravenously. Blood sugar before operation 46. At beginning of experimental period, 31; at end, 38.

Muscle glycogen at beginning 595 mgm. per cent; at end, 362 mgm. per cent. Blood lactic acids not done.

Dog 5. Five days of phloridzin and fasting.

Hepatectomy only. Weight at operation 11.8 kilos.

In 3 hours and 20 minutes received 4,350 grams glucose intravenously.

During this time excreted 1,000 mgm. glucose.

Used 3,350 glucose.

Blood sugar before operation 54. At beginning of experimental period, 51; at end, 68.

Muscle glycogen at beginning 219 mgm. per cent; at end, 132 mgm. per cent.

Blood lactic acid at beginning 65 mgm. per cent; at end, 63 mgm. per cent.

Urine contains 10 mgm. lactic acid.

DISCUSSION. The results of these experiments suggest that the tissues of the phloridzinised dog utilize glucose even when the blood sugar is kept low. Can we say that it is oxidized? It has not gone into extra muscle glycogen since the value regularly dropped. The blood lactic acid (and with it the tissue lactic acid) usually rose, but on the average, the amount of lactic acid that this corresponds to, together with that excreted in the urine, is about equivalent to the loss in muscle glycogen.

The average glucose utilization for all our animals amounted to 75 mgm. per kilo per hour or 1.8 gram per kilo per day. This represents something like 10 per cent of the metabolic needs of the animals. It is of interest to calculate the D:N ratios of our dogs when this amount of glucose is added to that which is excreted. Our dogs when completely phloridzinised (before the operation) had an average D:N ratio of 3.89 and excreted 3.07 grams glucose, and 0.808 gram nitrogen, per kilo per day. If we add the amount of glucose used by the tissues as calculated above (1.8 gram) to that excreted, we obtain the sum of 4.87 grams which when divided by 0.808 gram gives a D:N ratio of 6.04. If the conversion of fatty acids to glucose be denied, this would require an almost complete conversion of protein to

glucose (in contrast to the conventional 58 per cent). Some of this extra glucose may come from the glycerol fraction of the fats, though at most probably not more than one-third of this extra glucose could arise from this source.

On the other hand our results give no support to the concept that practically all foodstuffs, including fatty acids, are converted to glucose by the liver before utilization by the tissues. The glucose which we had to supply after hepatectomy represents a small fraction of the metabolic needs of the animal. The work of Bollmann, Mann, and Magath (1926) indicates that little if any of the energy turnover of liverless animals comes from protein. We are forced to the conclusion that most of the energy supply in our animals was from the direct utilization of fats.

SUMMARY

Completely phloridzinised dogs (3 to 5 days fasting and phloridzin) after hepatectomy require the administration of glucose to maintain the blood sugar level existing just before operation. The amount required averages 75 mgm. per kilo per hour. Analyses for lactic acid and muscle glycogen do not account for this glucose requirement.

If this glucose utilization by the tissues of the phloridzinised dog be added to that excreted, we obtain D:N ratios of the order of 6.

REFERENCES

- BOLLMANN, J. L., F. C. MANN AND T. B. MAGATH. *This Journal* **78**: 258, 1926.
DEUEL, H. J., JR., H. E. C. WILSON AND A. T. MILHORAT. *J. Biol. Chem.* **74**: 23, 1927.
DRURY, D. R. *J. Exper. Med.* **49**: 759, 1929.
MARKOWITZ, J. AND S. SOSKIN. *Proc. Soc. Exper. Biol. and Med.* **25**: 7, 1927.
WENDEL, W. B. *J. Biol. Chem.* **102**: 47, 1933.
WIERZUCHOWSKI, M. *J. Biol. Chem.* **73**: 445, 1927.

OBSERVATIONS ON THE BLOOD FLOW AND GASEOUS METABOLISM OF THE LIVER OF UNANESTHETIZED DOGS¹

ALFRED BLALOCK AND MORTON F. MASON

From the Departments of Surgery and Biochemistry of Vanderbilt University

Received for publication July 3, 1936

The flow of blood through the liver has been determined by a number of observers who have worked on anesthetized animals. Particularly pertinent among these studies are those of Schmid (1908), Burton-Opitz (1910, 1911), Barcroft and Shore (1912), Grab, Janssen and Rein (1929) and Schwiegk (1932). Schmid determined the flow of blood through the portal vein of deeply anesthetized dogs and cats by the use of a stromuhr. The measurements by Burton-Opitz were obtained after connecting a recording stromuhr with the hepatic artery in some experiments and with the portal vein in others. In these experiments, on anesthetized dogs, the portal vein was occluded for approximately ten minutes while connections were made to the stromuhr. Barcroft and Shore used anesthetized cats in their studies. The inferior vena cava was occluded between the adrenal veins and the hepatic veins and the flow was diverted into the jugular or brachial vein. Cannulae were then placed in the inferior vena cava above the point of occlusion and in a side branch of the portal vein and the flows through these two vessels were determined following temporary constriction. From these measurements, together with oxygen analyses, the gaseous metabolism was calculated. The average arterial blood pressure at the time of the measurement of the hepatic blood flow in Barcroft and Shore's experiments was 78 mm. mercury. Grab, Janssen and Rein working with anesthetized dogs used the thermoelectric stromuhr. No blood samples were taken for gas analyses. Schwiegk also employed the thermoelectric stromuhr on anesthetized dogs in determining the hepatic blood flow.

The purpose of the present experiments was to determine the blood flow and gaseous metabolism of the liver of unanesthetized dogs. A modification of a method previously described by Mason, Blalock and Harrison (1936) for measuring the renal blood flow plus the use of an instrument described by Horine (1928) made the study possible.

¹ Aided by a grant to Vanderbilt University from the Division of Medical Sciences of the Rockefeller Foundation.

METHOD. Dogs were used as the experimental animals in all instances. They were trained to lie quietly upon the table during the observation period. No food had been allowed for 18 hours preceding the experiment. No anesthetic was used except novocain locally at the sites of incisions in the neck. Aseptic technique was employed in the experiments in which additional studies were to be performed on subsequent dates. The animals gave no evidence of pain during the experiments and were observed to eat, play or fight following the completion of the studies.

The principle of the method consisted of producing a temporary blockage of the inferior vena cava both above and below the entrances of the hepatic veins and diverting the blood during this brief period into a cannula which had been inserted through the right external jugular vein. The cannula was made of brass and was 45 cm. in length. The distal end (liver) was closed and the proximal end (neck) was left patent. Slightly proximal to the distal end the cannula was equipped with two externally expanding balloons separated by a distance of 7.5 cm. These balloons (made from Penrose tubing) could be inflated by means of small internal tubes passing to the proximal end of the cannula. Between the balloons the cannula was perforated by fifteen large openings, leaving only enough cannula wall to render it rigid. The cannula had a capacity of approximately 1000 cc. of blood per minute at a level of minus 30 cm. At a preliminary operation, a number of days previously, an instrument described by Horine and designed to permit withdrawal of blood from the portal vein without surgical exposure was implanted. This device may be left in place for an indefinite period and apparently affords no discomfort to the animal nor does it interfere with the portal circulation. At the same time an untied ligature was placed around the hepatic arteries. The two ends of the ligature were brought out through the anterior abdominal wall.

At the beginning of the experiment, with the dog lying on his back, novocain was introduced over the sites of the two external jugular veins. Each vein was exposed and a short cannula, directed towards the heart, was introduced on the left. This was followed by the intravenous injection of 200 mgm. of heparin (Connaught Laboratory). The cannula equipped with the balloons was then introduced into the right external jugular vein and was passed through the superior into the inferior vena cava until its tip approximated the entrances of the renal veins. The proximal end of the cannula was closed during this procedure which was followed by aspiration of the air within the lumen of the tube.

With the aid of a fluoroscope, the cannula was then withdrawn until the opening into the tube closest to the uppermost balloon could just be seen above the diaphragm. From previous measurements, this signified that the uppermost balloon was between the entrances of the hepatic veins and the heart and the distal balloon was between the adrenal and hepatic

veins. This being true, the only blood which could enter the cannula when the balloons were inflated was that which came from the hepatic veins plus a small amount which came through the veins of the diaphragm. The cannulae in the jugular veins were connected by a T-tube with a side-arm of rubber tubing. This permitted through and through circulation.

After the cannula had been placed in correct position and the proper connections had been made, the rate of flow was determined. The lower balloon was inflated, the rubber tubing connecting the through and through circulation was occluded and simultaneously the blood was allowed to flow from the rubber tube side-arm into a calibrated vessel. This was followed immediately by the expansion of the upper balloon. After approximately 40 cc. of blood had escaped, the time required for the passage of 50 cc. of blood was determined. The observation was repeated a number of times with the outflow tube at different levels. The usual level was 30 cm. below the left auricle; however, this was governed by the capacity flow which was determined with the balloons uninflated. A position was chosen at which the observed flow was less than the capacity flow, but at which the pressure in the outflow system was not made sufficiently negative to cause marked pulsations due to collapse of the vena cava and hepatic veins. After satisfactory checks had been obtained, samples of blood for gas analyses were obtained. The hepatic vein sample was obtained from the tube through which the flow had been measured. Arterial blood was taken from the femoral artery and portal vein blood was drawn by introducing a needle through the instrument overlying the portal vein. All samples were taken with anaerobic technique. Approximately 200 cc. of blood were obtained from a donor prior to the experiment in order to have a store for replacement at the time of the measurements of the flow, and after sampling. It was injected into the left jugular vein at the time that the flow was being measured and at approximately the same rate. This kept the blood volume of the animal from being significantly altered at any time during the experiment. The oxygen contents, oxygen capacities and CO₂ contents of the blood from the three sources were determined.

Following the sampling procedure further measurements of the blood flow were made. The hepatic arteries were then occluded by pulling the ends of the ligature coming out of the abdominal wall, and 30 seconds later the flow was again measured. The difference in flow before and after occlusion was taken to represent that portion of the flow contributed by the hepatic artery.

In calculating the oxygen consumption of the liver the oxygen content of the hepatic vein blood was corrected on the basis of the oxygen capacity difference between it and the arterial and portal vein blood according to the method employed by Van Slyke et al. (1934). Eventually most of the dogs were sacrificed in order to obtain the weights of the livers. In some

TABLE 1

Hepatic blood flows and oxygen consumptions of dogs in which the hepatic arterial contribution to total flow was determined

DOG	WEIGHT	CORRECTED OXYGEN CONTENTS			A-V (O ₂)		CARBON DIOXIDE CONTENTS			APPARENT RESPIRATORY QUOTIENT
		Arterial	Portal vein	Hepatic vein*	Arterial hepatic vein	Portal vein; hepatic vein	Arterial	Portal vein	Hepatic vein	
	kgm.	vols. per cent	vols. per cent	vols. per cent	vols. per cent	vols. per cent	vols. per cent	vols. per cent	vols. per cent	
36	13.71	16.42	11.76	8.52 8.16	7.90	3.60				
26	18.79	20.57	16.04	11.47 11.38	9.10	4.66	39.80	43.15	45.90	0.61
26	18.50	15.57	11.20	6.45	9.12	4.75	41.68	45.75	49.14	0.74
17	16.10	18.00	12.43	5.04 5.02	12.96	7.41	36.20	42.82	46.44	0.52
17	15.82	13.93	6.28	4.65 4.58	9.28	1.70	31.14	36.82	40.18	1.36
195	13.20	13.28	6.47	4.12 4.29	9.16	2.18	39.98	43.68	45.50	0.70
27	14.28	15.55	9.38	5.03 4.79	10.52	4.59	37.04	42.62	44.56	0.53

DOG	WEIGHT, LIVER	HEPATIC BLOOD FLOW					PER CENT TOTAL FLOW FROM HEPATIC ARTERY	HEPATIC OXYGEN CONSUMPTION		
		Total	Hepatic artery	Portal vein	Per kgm. dog	Per kgm. liver		Per dog	Per kgm. dog	Per gram liver
	grams	cc. per minute	cc. per minute	cc. per minute	cc. per minute	cc. per minute		cc. per minute	cc. per minute	cc. per minute
36	553	476	84	392	34.7	0.86	17.6	20.75	1.51	0.038
26	546	612	77	535	32.6	1.12	12.6	31.94	1.70	0.058
26	546	462	70	392	25.0	0.85	15.2	25.00	1.35	0.046
17	535	313	48	265	19.4	0.58	15.4	25.85	1.61	0.048
17	535	385	88	297	24.3	0.72	22.9	13.22	0.84	0.025
195	482	487	119	368	36.8	1.01	24.5	18.92	1.43	0.039
27		469	99	370	32.8		21:1	27.40	1.92	

* The observed hepatic venous content is corrected in one case for the oxygen capacity difference between arterial and hepatic vein blood, and in the other for the difference between the portal and hepatic vein blood. Thus to calculate the A-V (O₂) there are two values for hepatic venous oxygen content.

instances this immediately followed the experiment and the position of the cannula was checked at autopsy.

RESULTS. The complete observations on total flow, flow following constriction of the hepatic artery, and gaseous metabolism were made on five dogs. In another series of animals similar observations were made except that the occlusion of the hepatic artery was omitted. In a further series the total flow and the flow after hepatic arterial constriction were

TABLE 2

Hepatic blood flows and oxygen consumptions of dogs in which the hepatic arterial contribution to total flow was not determined

DOG	WEIGHT	LIVER WEIGHT	CORRECTED OXYGEN CONTENTS			A-V (O ₂)		HEPATIC BLOOD FLOW			HEPATIC OXYGEN CONSUMPTION*		
			Arterial	Portal vein	Hepatic vein†	Arterial hepatic vein	Portal vein; hepatic vein	Total	Per kgm. dog	Per gram liver	Per dog	Per kgm. dog	Per gram liver
	kgm.	grams	vols. per cent	vols. per cent	vols. per cent	vols. per cent	vols. per cent	cc. per minute	cc. per minute	cc. per minute	cc. per minute	cc. per minute	cc. per minute
17b	14.20		20.04	16.59	12.10 12.04	7.94	4.55	438	30.8		22.91	1.61	
55	14.20	579	20.52	15.20	6.88 6.72	13.64	8.48	266	18.7	0.46	25.28	1.78	0.044
56	15.56		18.86	13.65	5.04 5.17	13.82	8.48	342	22.0		32.63	2.10	
6	11.50		18.17	16.73	11.40 11.77	6.77	4.96	344	29.9		18.31	1.59	
4	12.25	433	15.05	7.06	2.62 2.63	12.43	4.43	319	26.0	0.74	19.25	1.57	0.044
4	12.31	433	13.64	10.61	5.41 5.35	8.23	5.26	484	39.3	1.12	28.35	2.30	0.065

* Calculated on the basis of the hepatic arterial flow amounting to 20 per cent of the total flow. This is the average of 10 observations.

† See footnote table 1.

determined, but no blood samples were taken. Finally a number of animals were used for the purpose of determining only the total flow.

The results of the observations on those dogs in which the complete hepatic blood flows as well as the gaseous metabolisms were determined are given in table 1. In table 2 the results are given for the series in which the occlusion of the hepatic artery was omitted. The oxygen consumptions in these instances were calculated on the basis of the hepatic arterial

contribution to total flow being the average of all experiments in which this was determined, some of which are given in table 1.

DISCUSSION. *Total liver blood flow.* The total hepatic vein outflow was determined in 24 instances. The weights of the dogs varied from 11.5 to 18.8 kgm. and the liver flow varied from 216 to 612 cc., the average being 387 cc. The flow per kilogram of body weight varied from 17 to 39.3 cc., the average being 27.0 cc. In the instances in which the weight of the liver was determined, the flow per gram of liver tissue per minute varied from 0.46 to 1.12 cc., the average being 0.82 cc. Eight of the 15 values fell between 0.72 and 0.86 cc.

These rates of blood flow are much greater than those of Barcroft and Shore and are of the order of those obtained by Burton-Opitz, by Grab, Janssen and Rein and by Schwiegk.

Hepatic arterial blood flow. The hepatic arteries were constricted in ten instances, repeat determinations being performed on two dogs at intervals of several days. The reduction in the hepatic vein flow which resulted from this procedure varied from 12.6 to 24.5 per cent of the total, the average being 19.5 per cent. Conversely, the flow of blood through the portal vein constituted 80.5 per cent of the total liver flow.

In fasting animals, Barcroft and Shore found that the hepatic arterial flow constituted 34 per cent of the total flow through the liver. Burton-Opitz did not determine the portal vein flow and the flow through the hepatic artery in the same animal but concluded from a number of studies on each that 30 per cent of the total quantity of blood passing through the liver came from the hepatic artery. Grab, Janssen and Rein found that the average contribution of the hepatic artery was 19.0 per cent of the total liver flow, which is in close agreement with our findings. The variations we have observed in this function are also of the order observed by those authors and by Schwiegk.

Constriction of the hepatic arteries resulted in a marked diminution in the oxygen content of the blood in the hepatic veins. In several instances the values were less than one volume per cent during the occlusion period.

As has been stated, the portal flow through the liver was measured 30 seconds following the occlusion of the hepatic arteries. Measurements made several minutes later with the constriction still maintained usually showed that the flow had returned to the pre-occlusion rate.

Oxygen consumption of the liver. The total oxygen consumption of the liver varied from 13.2 to 32.6 cc. per minute, the average of all experiments being 23.8 cc. per minute. The oxygen consumption per gram of liver per minute varied from 0.025 to 0.065 cc., the average being 0.045 cc.

Barcroft and Shore found that the oxygen consumption of the livers of anesthetized cats which were fed 18 hours before the experiment varied

from 0.024 to 0.050 cc. per gram per minute and that the hepatic artery was the dominating source of oxygen supply to the liver.

Schwiegk, in experiments on dogs anesthetized with chloralose, obtained portal vein blood oxygen unsaturations of about 30 per cent and hepatic vein unsaturations of about 50 per cent, and concluded that on the average the hepatic artery supplied about 40 per cent of the oxygen utilized by the liver. In the series of experiments for which the data are given in table 1, the hepatic artery supplied between 22 per cent and 38 per cent of the oxygen in five instances and 58 per cent and 62 per cent in two others. All these dogs were presumably in the fasting state.

Respiratory quotient. In those cases where the hepatic arterial contribution to the total flow was actually determined (table 1) the respiratory quotients were computed. The values obtained were variable, and it is doubtful if any interpretation can be made of them; however, in some preliminary experiments constant gross changes were noted after administration of food, and this point is being further investigated.

The method which has been described has certain advantages as a means of studying hepatic physiology. It affords the opportunity of obtaining relatively large samples of hepatic vein, portal vein and arterial blood simultaneously from the unanesthetized animal and at the same time permits the determination of the portal and arterial supply in relation to the total flow. It has not been satisfactory as a means of studying the transient or rhythmic changes in the blood supply of the liver for which purpose the thermo-electric stromuhr is ideally suited.

SUMMARY

The hepatic oxygen consumption and the total, portal, and arterial blood flow through the liver of the unanesthetized dog have been determined.

REFERENCES

- BARCROFT, J. AND L. E. SHORE. J. Physiol. 45: 296, 1912.
BURTON-OPITZ, R. Quart. J. Exper. Physiol. 3: 297, 1910; 4: 113, 1911.
GRAB, W., S. JANSSEN AND H. REIN. Ztschr. Biol. 89: 324, 1929.
HORINE, C. F. Arch. Surg. 17: 289, 1928.
MASON, M. F., A. BLALOCK AND T. R. HARRISON. J. Biol. Chem. (Proc.) 114: lxiv, 1936.
SCHWIEGK, H. Arch. f. exper. Path. u. Pharmacol. 168: 693, 1932.
SCHMID, J. Pflüger's Arch. 125: 527, 1908.
VAN SLYKE, D. D., C. P. RHOADS, A. HILLER AND A. S. ALVING. This Journal 109: 336, 1934.

THE DISTRIBUTION OF GLUCOSE IN BLOOD

ISAAC NEUWIRTH

*From the Department of Pharmacology and Therapeutics, College of Dentistry,
New York University, New York*

Received for publication, July 3, 1936

Olmsted (1) has reported that when proper precautions are taken the blood cells of various species, including man, contain little or no glucose. All of the glucose is in the plasma. He (2) has further stated that the blood cells of various species differ in their permeability to glucose after the addition of oxalate to the blood. In this respect the corpuscles of man are least resistant to and more readily disturbed by the addition of this anticoagulant. This was demonstrated by a study of sugar distribution in oxalated blood at various time intervals.

These findings of Olmsted are at variance with those generally accepted regarding blood sugar distribution. They are of importance in connection with many physiological investigations, for example, those on kidney secretion.

A reinvestigation of the above work (2) using blood as ordinarily drawn with oxalate as the anticoagulant, failed to confirm Olmsted's findings that human blood cells readily become permeable to glucose, in contrast to rabbit blood cells, which are reported to do so slowly. In twelve experiments using human blood, in which the sugar distribution was studied up to three hours, and in three experiments with rabbit blood, in which the sugar distribution was studied up to one and one-half hours, there was no evidence of an increased penetration of sugar into the cells. However, in five experiments with pig blood, in which the sugar distribution was studied up to eight hours, the findings of Olmsted were confirmed.

Our next experiments were carried out using the special precautions outlined by Olmsted. Here again, in the case of human and rabbit blood, his findings (1, 2) were not confirmed. Our results are given in table 1. It should be pointed out that the determination of whole blood sugar was done only on the oxalated material.

The methods used for the determination of glucose were those of Benedict (3, 4). These methods are as specific for glucose as those used by Olmsted. A problem of this nature fundamentally resolves itself down to a determination of differences in sugar content of oxalated and unoxalated (no anticoagulant) plasmas. No difference in glucose content in such plasmas was found in this work (table 1).

TABLE 1

Milligrams of glucose in plasma and corpuscles given as in 100 cc. of whole blood

HEMATOCRIT	WITHOUT ANTICOAGULANT		OXALATED		AFTER OXALATE*	
	Plasma	Corpuscles	Plasma	Corpuscles	Plasma	Corpuscles
Human						
<i>per cent</i>						
42	55	32	55	32		
48	48	39	48	39		
44	51	34	52	33		
50	33	29	32	30		
42	52	30	51	31		
49	41	33	40	34		
50	44	36	43	37		
46	50	36	50	36		
45	44	31	45	30	41	28
47	41	31	41	31	38	29
42	50	30	50	30		
41	47	27	47	27	44	24
46	50	36	51	35	48	34
47	48	37	48	37	46	35
46	49	33	49	33	45	32
45	57	38	57	38	53	36
43	60	39	60	39	57	36
46	44	32	44	32	41	30
Rabbit						
38	104	5	102	7		
35	91	13	90	14		
35	120	17	119	18		
37	52	12	51	13	44	8
37	88	17	87	18	76	13
30	50	9	49	10		
36	116	19	115	20	103	15
31	117	11	118	10	102	8
31	97	14	97	14	90	11
34	104	11	103	12		
31	110	14	109	15	96	10
37	147	20	147	20	139	16

* For human bloods, 30 minutes; for rabbit bloods, 75 minutes.

CONCLUSION

The findings of Olmsted that human and rabbit blood corpuscles contain no glucose are not confirmed. Nor are his findings that oxalate changes the permeability of such blood cells to glucose verified.

For drawing the samples of human blood, I am indebted to Doctors Brown and Lane; for the rabbit blood, to Mr. J. F. Reinhard. I am also grateful to those students from whom blood was obtained.

REFERENCES

- (1) OLMSTED, J. M. D. This Journal **111**: 551, 1935.
- (2) OLMSTED, J. M. D. This Journal **114**: 488, 1936.
- (3) BENEDICT, S. R. J. Biol. Chem. **76**: 457, 1928.
- (4) BENEDICT, S. R. J. Biol. Chem. **92**: 141, 1931.

A COMPARISON OF THE ELECTROGRAM OF THE OPTIC CORTEX WITH THAT OF THE RETINA¹

S. HOWARD BARTLEY

*From the Laboratory of Neurophysiology, Washington University School of Medicine,
St. Louis, Mo.*

Received for publication May 18, 1936

Certain visual phenomena can be recorded not only from the eyeball itself in the usual electro-retinogram, but from other stations in the optic pathway such as the optic nerve (Adrian and Matthews, 1927) or single active fibres before they leave the eye as part of the optic nerve (Hartline, 1935). Further, it is possible to determine some aspects of retinal behavior by recording from the striate area of the cerebral cortex, for most of the latency of the cortical response represents time consumed in the eye (Bartley 1934). Stimulus conditions which vary the latency of retinal response effect quite similar changes in the cortical response. Certain comparative details of the two responses are presented to show the possibility of using the first wave in the cortical response for measuring activity as far back as the eye.

The typical retinal response to a long flash of light is as in figure 1A. To the beginning of the flash a wave complex occurs called the *on* response, and to the cessation another called the *off* response. The *on* is composed of the *a* and *b* waves, the *a* not appearing in the records of all animals or of all preparations. If the stimulus is long, a subsequent gradual development of potential may occur (the *c* wave), and if the flash is weak or short no *off* response (*d* wave) appears. Several experimental procedures, such as asphyxiation, etherization, temperature manipulation, and massage of the eyeball markedly alter the shape of the whole retinal record, a result which has led to the analysis of the picture into three components, PI, PII and PIII (Granit, 1933, modified after Piper, 1911). Our interest is in the *b* wave of the *on* effect, which Granit believed to be produced by PII, the only one of the three processes concerned with the discharge of impulses into the optic nerve; and also in the possible *off* effect. It is the *b* wave of the retinal record that compares most closely with the cortical response, but since the cortical record exhibits an *off* effect, its precursor might be expected in retinal neural action. Granit, however, attributes

¹ This study was made under a grant from the Rockefeller Foundation for Research in Neurophysiology.

the retinal *off* effect to a rapid return of the negative PIII wave to the base line and has excluded PIII from connection with neural discharge, except as an inhibitor of PII. From this, no cortical *off* effect might have been expected, but on the contrary it is far more pronounced than the retinal *off* response, which findings suggest a reinterpretation of some of the findings of the electro-retinogram technique. The finding of a cortical *off* response is in keeping with those of Hartline mentioned below, in which single afferent fibres which are to compose the optic nerve exhibit an *off* response.

It will be noted that all of the transitions in the retinal record are slow, so the components of the *b* wave are long. It is possible that the record includes not only neural potentials but other processes, though it does not

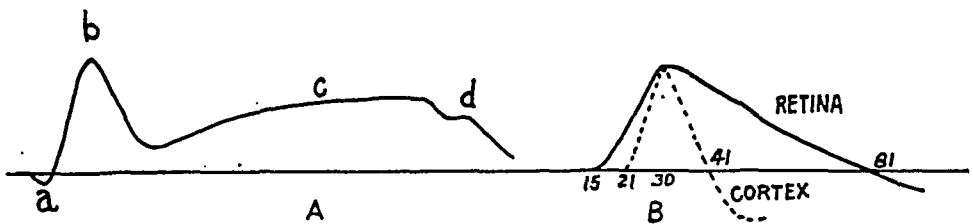


Fig. 1. A. Shows a typical response to a flash of light a few seconds long recorded from the eyeball. In some animals a small negative deflection, the *a* wave, appears first. The *b* wave occurs in all, and under appropriate conditions the slow *c* wave. If the light lasts long enough and is bright enough a further small deflection in the general course of the potential appears (the *d* wave) at the cessation of the light. In all cases the potentials are slow and do not reveal the complicated events which we know are occurring in the eye.

B. A comparison of the time relations of the *b* wave of the retinal record and the first potential of the cortical response. The onset of the *b* wave is often very gradual but definitely precedes the onset of the cortical wave. Their peaks may coincide, however. Some difficulty is experienced in determining the exact peak of the *b* wave, for it is somewhat broad. Here again the relative abruptness of the cortical response is demonstrated.

seem to be readily analyzed in ways essentially different from the method already described.

The optic nerve record, like the retinal, portrays the gross activity of the nerve, and for analysis, ways must be sought to divide the nerve into its anatomical constituents, the fibres. This in effect has been done in Hartline's experiments in recording from afferent fibres just before their collection into the optic nerve. By this technique he has been able both to record discrete discharges in single fibres and to study the activity of local retinal areas. The repetitive end organ discharge which is recorded in the optic nerve is rearranged as it reaches the first way station, the lateral geniculate body. Facilitation in this nucleus condenses the afferent discharge which arrives at the onset of activity (Bishop, 1933; Bartley,

1935) so that the cortical response is a more abrupt and shorter wave than the retinal *b* wave, a result working to advantage in many experiments.

METHOD. All of the records were obtained by the use of the cathode-ray oscillograph and vacuum tube amplifier on rabbits held in light ether anesthesia by tracheal cannulae.

The flashes of light were regulated by a sector disc whose speed could be varied and measured, the latter by a Weston tachometer. The beam of light emerged from a narrow slit, so that the onset of the flash occupied only 1.5–8 sigmas depending on the speed of the disc. The light illuminated the face of a biconvex lens whose face subtended a visual angle of $11^{\circ}20'$. The visual angle concerned us only in the production of a large flux of light and not in any direct spatial way, for it has been shown that both retinal and cortical records are the result, for the most part, of the activity of the stray light illuminating the retina in general (Bartley, 1935; Fry and Bartley, 1935). The maximum brightness was 30,500 c/ft² and unless otherwise specified was used in all experiments except the comparisons between the cortical *off* and *on* responses. The dim flashes were 0.004 of the maximum. In the comparisons between cortical *off* and *on* responses the visual angle was 7° and the brightness was 1280 c/ft². The pupillary aperture in each case was in the neighborhood of 15 sq. mm. Some light also fell on the cornea outside the pupil.

EXPERIMENTAL. The following experiments give a comparison of the gross features of the retinal *b* wave (hereinafter called the retinal record or retinal *on* effect) and the cortical record and show how the two responses behave under the same manipulation of stimulus conditions.

1. *Comparison of gross time relations.* If a 115° flash once every 1800° is presented, the *peaks* of the retinal and cortical *on* effects coincide in time. In one animal, for instance, they were both at about 30° , the retinal effect of course *beginning* sooner and *ending* much later than the cortical. The starts of the two waves were 15° and 21° respectively for retina and cortex, and the completions of their positive phases 81° and 41° (fig. 1B). Inasmuch as both effects are diphasic, these last values do not mark the completion of the whole effect. Flashes which approach the critical duration shorten the responses a little. And, too, the onset of the flash may be made gradual enough to practically obliterate the *on* response as a definite wave in the record. The difference in results with short and long stimuli will be brought out more definitely in the next experiments to be described.

2. *Reduction of amplitude and increase in latency.* Among the factors that will decrease the amplitude and increase the latency of response is the repetition of the flash at short intervals. This is done by simply increasing the speed of the sector disc. Figure 2 shows the results of using various intervals between the origins of the flashes, ranging from 1800°

to 45° . Part of this range was covered both by flashes equal in length to the interval between them and by short flashes from 11 to 4° , a light-dark ratio of 0.006 as compared to 0.5000. The curves are from one set of experiments on a single animal; though the trend of the results is typical, the absolute values cannot be taken as more than representative. The points to be gained from the curves are that shortening the interval be-

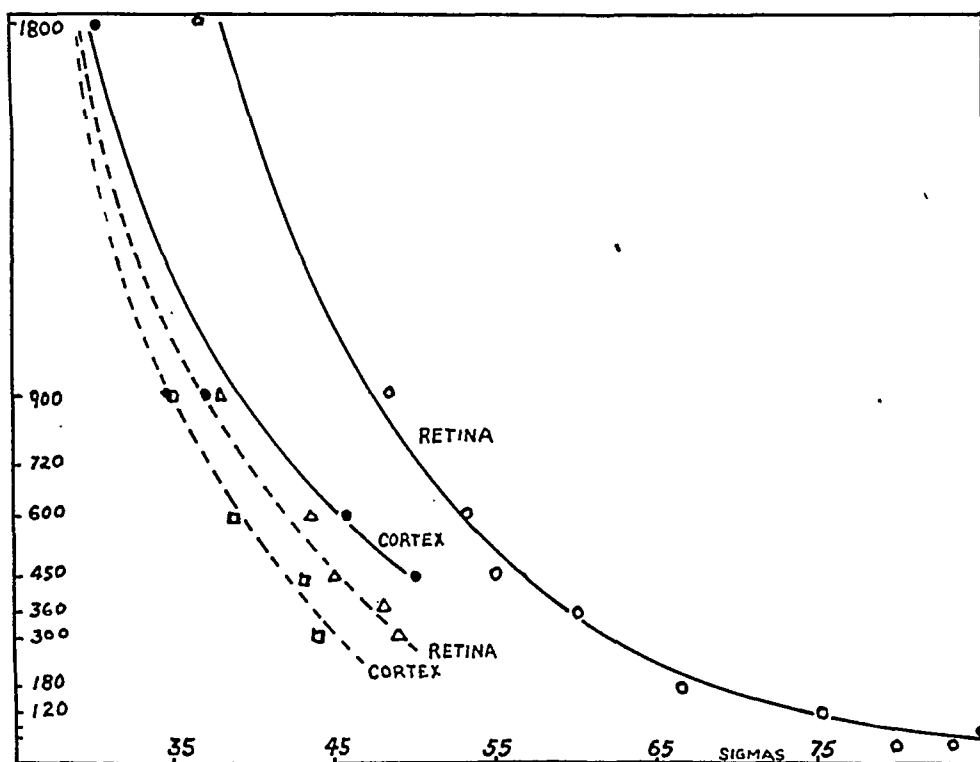


Fig. 2. The relation between flash intervals and the latency of the peaks of the retinal and the cortical records. The ordinate represents the flash intervals and the abscissa the latency, known as the "implicit time" in connection with cortical records when so measured. The solid lines are for cases of dark and light intervals of equal length. The broken lines are for short flashes (11- 4°) in which the light-dark ratio is 0.006 rather than the former 0.500. It will be noted that with short flashes the retinal and cortical peaks more nearly coincide. It is sufficient to see that the curves do not diverge greatly under similarly changing conditions as through some possible function of the geniculate station.

tween flashes increases the latency for both the retinal and cortical responses as measured to the peak of the waves. The peak of the retinal response is temporally shifted more than the cortical peak, though the two peaks often arise at about the same time with short flashes.

As the interval between the beginnings of the flashes is reduced from 1800° , the amplitude of both retinal and cortical response dwindles.

Figure 3 shows the manner in which the reduction occurs. Curve *a* represents the positive phase of the retinal response, and *b* both phases. Curve *c* represents the positive phase in another animal, using shorter intervals, and *d* the dwindling of the cortical response in the same animal. The curves *a* and *b* are measured from photographs, and *c* and *d* upon the face of the oscillograph. The curves indicate that the retinal response disappears with an interval between 45 and 30°, which in terms of flicker would represent a critical frequency of about 22 to 33.

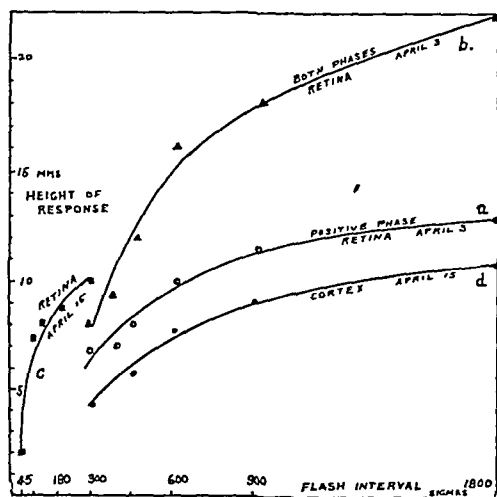


Fig. 3

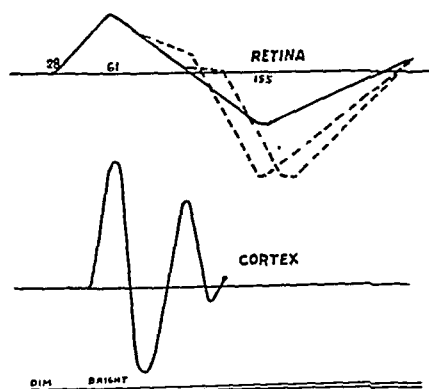


Fig. 4

Fig. 3. The relation of amplitude to flash interval. Repetition was chosen as one way to reduce amplitude to show that the reduction in the size of the cortical response and retinal responses is similar. The retinal response not having spontaneous potentials can be reduced to the vanishing point, whereas the cortical response can not, for when the response becomes small it is lost in the perpetual cortical activity whose waves are quite like the response itself. This is an experiment to show that the cortex responds to as high repetition frequencies as the retina.

Fig. 4. A comparison between cortical and retinal records when a two-stage flash is used as a stimulus. The first part (45°) was only 0.004 as bright as the second (25°). The retinal diagram shows also a response to a lengthened dim portion. Both the first and second responses in the retinal record are diphasic as evidenced by the increase in the negativity over the original wave to a homogeneous flash drawn in the solid line. Note that the cortical response is abrupt enough to avoid the overlapping shown in the retinal picture.

The curves also show that the cortical response dwindles in a manner similar to the retinal one. However, the former cannot be identified when it becomes small, on account of the spontaneous and also secondary response waves which mask it. The observer has therefore to be content with using the cortical response when it is large enough to stand out from the spontaneous activity and under conditions in which the secondary effects of two responses do not greatly overlap.

3. *Consecutive on responses.* A second stimulus may be given without the complication of intermediate *off* effect of the first by arranging the flash so that the first part of it is dim and the latter part bright with an abrupt transition between the two. A situation of this kind will be called one supplying two consecutive *on* stimuli. With such an arrangement a single response resembling the response to a homogeneous flash occurs in both the retinal and cortical records if the dim part of the flash is short. But by the time the dim segment is increased to $28-30^\circ$ (the bright 25° and flash interval of 900°), a second peak will have definitely begun to appear on the descending limb of the positive phase of the retinogram as in figure 4. The original flash 55° totally dim had elicited a wave beginning at 28° with its peak at 61° . The new hump has a latency of about the same order as the first one. If the dim segment is made still longer, the hump appears later, as expected. In both cases the swing of the negative phase below the baseline is accentuated, showing the essential diphasic nature of the retinal response (*b* wave).

Dim segments of 8 and 16° do not produce a second peak in the cortical record, but as in the retinal response, cause an increased latency over that of a flash, all of which was of the intensity of the bright segment. The delay for both the above values was equal (8°). When the dim segment was made 45° long, two definite responses appeared, without the overlapping of the positive phases which characterized the retinal record. This is shown in figure 4.

4. *Short dark intervals.* Short dark intervals interrupting an otherwise continuous bright light produce no effects in the retinal record. Granit and Riddell (1934) found only the faintest trace to a 22° dark interval with the illumination they used. A 100° dark interval presented once every 1800° produces only a slight response with our highest brightness. With a 35° interval no response is recorded, while with a 52° interval there is a trace.

On the other hand, the cortical record shows a definite response to both the beginning and ending of the interval; in other words, both a definite *off* and an *on* response. Under optimal conditions these appear with an interval as short as 12 sigmas.

Under none of the conditions used did our flashes or dark intervals produce a definite retinal *off* response. For conditions favorable to the retinal *off* response appearing in the retinogram, see Granit (1933).

5. *A comparison of the cortical on and off responses.* The fact alone that an *off* response appears at the cortex would suggest its existence as a neural phenomenon in the eye. But Hartline's finding of an *off* discharge in optic nerve fibres already had proved that the *off* response exists as a neural process there. If it fails to occur in the retinal record when it appears under similar conditions in the cortical record, it must be because

it is masked in the algebraic summation of it with other simultaneous processes and not for other reasons.

On account of the fact that the *off* response can be better isolated at the cortex, a comparison of cortical *off* and *on* responses follows.

The implicit time of the *off* response, like that of the *on*, can be varied by changing stimulus intensity. Figure 5A is a typical curve and is similar to that for the *on* response (Bartley, 1934). The response also varies considerably in size. These findings are contrary to the report of Wang (1934) in which it was stated that the size and latency of the *off* response does not vary, but in his case it is probable that the amount of

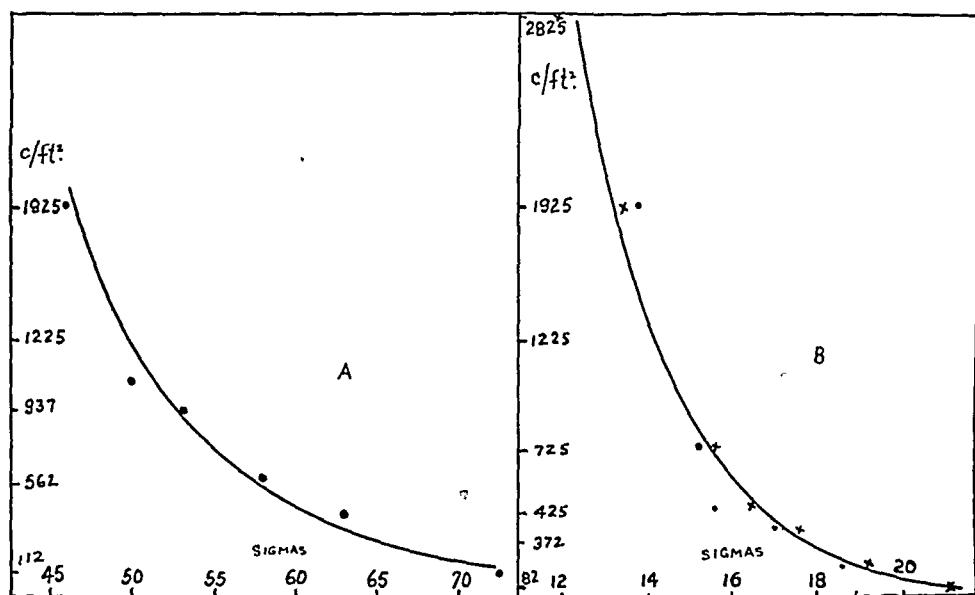


Fig. 5. A. The relation between flash intensity and the implicit time of the *off* response. Implicit time is the latency as measured to the peak of the response potential. This points out a similarity between the *off* and *on* responses.

B. The relation between intensity of flash necessary to produce an *off* response and the duration of the dark interval.

light used represented a level too high to produce a measurable response variation. The relation of the observable threshold of response to intensity and duration (fig. 5B) is a typical quantity curve, as was the threshold curve for the *on* effect, previously shown (Bartley, 1934).

The implicit times of the *on* and *off* responses of the cortex are not similar and may be compared by several kinds of experiments. If a constant illumination is interrupted by a dark interval, both an *off* and an *on* response will occur, in this case the *off* response, of course, preceding the *on*. The implicit times of both can be measured. If, on the other hand, a dark period is interrupted by a flash of light, both an *on* and an

off response may be observed, depending on the length of the flash. Comparing the implicit time of the *off* response for the beginning of the dark interval, and the *on* response for the flash, one will find that for the same intensity of illumination, the latency of the *off* response is much shorter. Or, comparing the implicit times of the *on* and *off* responses for a single flash or for a single dark interval, of similar duration, one will also find the implicit time of the *off* response shorter.

If light flashes are very short, no identifiable *off* response will follow the *on*.

On the other hand, if a dark interval is used, and the *off* response thus made to precede the *on*, the latter will follow the *off* very closely. Intervals as short as 12° may produce responses of nearly equal size to both the *off* and *on* (Bartley, 1936). The fact that the two types of responses can follow each other so closely suggests a more or less separate pathway clear to the cortex maintained by the differently stimulated fibres, for when similar pathways are activated a second time, no response will be elicited so soon after the first. That is, paired electric shocks to the optic nerve can not be spaced so closely and produce a response.

The *off* response, like the *on*, may exhibit at least a second wave following the first at a considerable distance. For example, when a dark interval is the stimulus, such as in the case above, three potentials can be observed to clearly rise out of the spontaneous activity. In a typical case, their peaks appeared at about 67, 115 and 162° respectively as measured from the *beginning* of the dark interval. Assuming the first to be the *off* response, its implicit time was 67° , and the third to be the *on* response, its implicit time was about 122° . In order to assign the second potential to the proper stimulus, the dark interval was lengthened by adding about 25° on to the beginning. In this case the three peaks appeared at 39, 86 138° as measured from the beginning of the original dark interval, which was the beginning of the line on the oscillograph. By adding 25° on to the first two values, they will approximately match the original readings. They will be 64, 111 and 163, as compared to 67, 115 and 162. From this, the second of the three responses belongs to the *off* effect and with an implicit time of about 110 to 115° .

Or, in another case in which the beginning of the oscillograph line was synchronized with the *end* of the dark interval, it being about 25° long, responses appeared at 48, 110 and 175° . Counting the first as an *off* response, 25° had to be added, making the implicit time 73° . The second wave was the *on* response (see below) with an implicit time of 110° . In this experiment the third wave is the second *on* response. It is possible that the second *off* response did appear in the record also, but we were unable to definitely identify it amidst the spontaneous variations occurring in the record or to dissociate it from the wave complex in the region

of 110° where it would likely have appeared. When 47° were added to the beginning of the dark interval, waves at 67° and 110 – 115° definitely appeared. The total dark period was now 72° long, bringing the first *off* response at or before the beginning of the oscillograph line. The first wave on the line (67°) was taken as the second *off* response, and the second as the first *on* response. The second *on* response also appeared part of the time. On other occasions when no succeeding *on* stimulation was introduced to complicate the situation, a wave has been observed to follow the *off* response at an interval of about 154° or more. The *on* responses under corresponding conditions also included a second wave in the neighborhood of 162° . It is probable that this wave in the two cases compares with the corresponding one to electric stimulation of the optic nerve, in which it has been measured as early as 164° (Bartley and Bishop, 1933).

The appearance in both cases of a second wave reveals another similarity in the general mechanism of the *off* and *on* responses. And its location identifies it with the second wave of the response to electrical stimulation.

DISCUSSION. The purpose of presenting the foregoing facts was to make clear the use of cortical implicit time as an index of retinal behavior in ways similar to the present use of the retinal *b* wave. Inasmuch as the cortical recording site is quite remote from the retina, it is natural that doubt should arise at first regarding the use of any measure of cortical latency to study retinal behavior. It happens, however, that the synaptic junction (lateral geniculate body) acts in a way to make this possible. The delay at this point is not great and facilitation there condenses the burst of impulses arriving first into a relatively short and abrupt wave appearing as the first part of the cortical response.

The experiments show that the cortical record follows the retinal in its essential changes due to varying stimulus conditions, especially if the peaks of the two waves are used as points of reference. The further useful feature of the cortical response is that it represents an analysis of the activity of two of the kinds of discharge patterns, referable to the two groups of fibres found by Hartline, namely, those fibres discharging with a burst at the onset of the light flash and those discharging at its cessation.

Furthermore, the *off* response when it occurs has all the essential features of an *on* response and must be regarded as occupying a different set of elements, which, however, behave in the same way as those responsible for the *on* response. This is at variance with the conception that the *off* response is simply a restimulation due to a release of PII from the inhibitory effect of PIII. Creed and Granit (1933) definitely state that PII alone is associated with the discharge of impulses along the optic nerve and the negative PIII is some process which tends to actively inhibit it. In their conception, the *off* discharge represents a post-

inhibitory rebound of the *b* process, by implication activating the same fibres as initially activated by this process. If to Hartline's finding of separate fibres activated at *off* and *on*, is added the present finding that the interval between an *on* and an *off* response of the cortex can be less than that possible between two *on* responses, the mechanisms of the *on* and *off* effects appear to be separate throughout the pathway, and no inhibiting process is demanded to account for them. A cortical response may appear, even when the envelope of the retinal record fails to show a separate elevation to account for it.

The change in form of the retinal record with the duration of illumination raises a further question as to the significance of its configuration. The diphasic character of the *b* response to a short stimulus suggests that the elements responding to cause the *b* elevation give individually diphasic responses. The apparent monophasicity of the response to a prolonged illumination must be a function of the particular summation envelope that prolonged stimulation of such (repetitive?) elements results in. The *off* response when it occurs might then also be interpreted as the index of similar activity in different elements, its second phase, if any, being observed in the general falling off of potential following the cessation of stimulation.

SUMMARY

Certain conditions were selected in which to compare the *b* wave of the retinal record with the first wave of the cortical response. The following are the results.

1. The cortical response is more abrupt than the retinal and the possibility of its revealing certain things that may be masked in the retinal record is shown.

2. The two responses parallel each other rather closely in their latency changes with changing conditions.

3. They parallel each other rather closely in their reductions in size when the flash rate is increased, but the cortical response can not be used when small.

4. The cortical response shows a definite *off* response when no such wave is evident in the retinal record.

5. The behavior of the cortical *off* response is at variance with the concept that the *off* response is a post-inhibitory rebound, by implication involving the same optic nerve elements as the *on* response.

REFERENCES

- ADRIAN, E. D. AND R. MATTHEWS. J. Physiol. 63: 378, 1927.
BARTLEY, S. H. AND G. H. BISHOP. This Journal 103: 159, 1933.

BARTLEY, S. H. This Journal 108: 397, 1934.

This Journal 110: 666, 1935.

J. Cell. and Comp. Physiol. 8: 41, 1936.

CREED, R. S. AND R. GRANIT. J. Physiol. 78: 419, 1933.

FRY, G. A. AND S. H. BARTLEY. This Journal 111: 335, 1935.

GRANIT, R. J. Physiol. 77: 207, 1933.

GRANIT, R. AND L. A. RIDDELL. J. Physiol. 81: 1, 1934.

HARTLINE, H. K. Proc. Am. Physiol. Society, This Journal 113: 59, 1935.

PIPER, H. Arch. J. Physiol., p. 85, 1911.

WANG, G. H. Chinese J. Physiol. 8: 121, 1934.

RESPIRATORY REACTIONS UPON VERTICAL MOVEMENTS¹

E. A. SPIEGEL

From the Department of Experimental Neurology, D. J. McCarthy Foundation, Temple University, School of Medicine, Philadelphia, Pa.

Received for publication February 8, 1936

The vegetative effects of labyrinthine reflexes upon vertical movements seem of importance in seasickness and airsickness. Little is known, however, about such reflexes. Graham Brown (1904) found in frogs that up and down movements induce first an increase of the intrapulmonary pressure. In the second part of the reaction movements of the lungs were missed, and only slight oscillations of the intrapulmonary pressure were noticed, that were probably due to slight contractions of the wall of the lungs. Pozerski (1921) was unable to produce symptoms of seasickness with his rocking apparatus in mammals such as guinea pigs, rabbits, even if they were exposed to the movements of this machine for 6 hours. Yet in 30 per cent of the dogs vomiting, polypnea, pollakisuria appeared. Sjöberg (1931) could provoke the typical symptoms of seasickness (increased salivation, polypnea, diarrhea, polyuria, psychic agitation, later apathy, vomiting) with all dogs under study when the animals were exposed to rapid up and down movements in a crane. The question, however, arises how far these symptoms are reflex phenomena due to labyrinthine or other reflexes upon the vegetative system, and how far they are secondary to the emotional excitation. Such an analysis seems particularly desirable in regard to the respiratory changes in mammals, since the anxiety and unrest of the seasick animals can produce by themselves respiratory reactions. The present investigation deals with an analysis of the respiratory reactions in vertical movements.

METHOD. Pitching movements were imitated by a rocking board upon which the animals (cats and dogs) were fastened. (Distance of the rotation axis from the end of the board, $1\frac{1}{2}$ m., vertical distance of the highest and of the lowest position, 97 cm.) The rocking movements were produced by a motor with a variable frequency of 18 or 36 up and down movements per minute. Purely vertical movements were produced by connecting the end of the rocking board through a pulley with a horizontal animal holder that slid up and down between two vertical bars. Special care was taken to avoid accessory movements of the animal board, particularly

¹ Aided by a grant from the Ella Sachs-Plotz Foundation.

concussion, since such movements may by themselves produce respiratory or vasomotor reactions. One has further to prevent the effect of inertia. In the turning points, the animal's body tries to continue its movement, while the movement of the board is already reversed. This plays a rôle particularly, when the downward movement is changed into an upward movement, since the impact of the board against the thorax may induce reflexly respiratory reactions. Such reactions are particularly noticeable if the animal is in prone position; they were avoided by fastening the animal in supine position and fixing the head in its holder in such a way that the upper thoracic part of the back did not touch the board. It was established that the noise of the motor did not produce any respiratory reaction. The respiration was recorded by a Harvard pneumograph fastened around the chest or by connecting a Y shaped cannula inserted into the trachea with the recording tambour. The up and down movements were recorded by a signal magnet connected with an electrical switch that was automatically closed at the lowest position of the animal.

RESULTS AND COMMENT. Both types of movements, the purely vertical as well as the pitching movements, produced an increase of amplitude and frequency of respiration in unanesthetized animals. This effect was observed in dogs and cats already in the beginning of the oscillations; it was even often most pronounced at the start, while vomiting appeared in dogs much later, if at all, and did not appear in cats. Sjöberg also observed in his dogs the vomiting only 11 to 30 minutes after the beginning of the vertical movements. Yet a single movement or 2 or 3 up and downward movements may be able to elicit a respiratory reaction; the downward movement proved to be somewhat more effective than the upward movement. When the animals were continuously exposed to these vertical oscillations, some showed a decrease of the reaction after a few minutes, in others the change in respiration was sustained for ten and more minutes. Not infrequently the amplitude of the respiratory movements increased and decreased periodically in slow waves of $\frac{1}{4}$ to $1\frac{1}{2}$ minute duration.

The increase of the amplitude affected inspiration and expiration, the former effect appearing on the records of the thoracic respiration, the latter reaction appearing particularly on the graphs taken from the trachea. After the vertical movements were stopped, the amplitude of the respiratory movements was often diminished as compared to the respiration of the resting animal before the elevator movements had started. In other cases, however, an afterreaction outlasting the stimulation could be observed. Under superficial anesthesia (ether, dial-ether, morphine-ether anesthesia), the increase in the rate of respiration became less pronounced, while the increase in amplitude was still distinct. Under deep anesthesia the change in frequency as well as in amplitude subsided.

Effect of removal of higher centers. Typical respiratory reactions, such as

initial increase of the respiration, waves of increase and decrease in amplitude, were still observed after extirpation of both cerebral hemispheres (fig. 1, B). Extirpation of the hypothalamus in addition to removal of the cerebral hemispheres with the optic thalamus proper also did not abolish this reaction. When a transverse section behind the midbrain was performed, the respiratory reactions were markedly diminished, but not completely abolished. The conclusion seems justified that the respiratory changes induced by vertical movements are only partly due to psychic excitation, that, besides this factor, brain stem reflexes play an important part. In a few decerebrate animals an inhibition of the respiration (diminution of the amplitude) was noticed at the beginning of the movements. Such a reaction was particularly observed, when the respiratory center was in a poor condition, e.g., due to loss of large quantities of blood (fig. 1, D).

Receptors. Absence of *optical* impulses does not prevent the above mentioned respiratory reactions, as shown after severance of the brain stem behind the entrance of the optic tract into the external geniculate body. This, however, does not exclude the possibility that impulses from the retina may participate in the production of these reactions. The influence of vertical movements was, therefore, studied before and after the eyelids were closed by a tape. This had no definite effect upon the respiratory reactions on otherwise normal animals. If, however, more effective receptors were already eliminated (doublesided labyrinthectomy, transverse section of the cord), the remaining respiratory reactions on vertical movements were sometimes slightly diminished, when the eyelids were closed. Optical impulses seem, therefore, to have but a slight contributory influence in the stimulating effect of vertical movements upon respiration.

Severance of the *trigeminal* nerves in decerebrate animals did not change the respiratory reactions. The result was the same in animals with intact labyrinths as in animals with paralyzed labyrinths.

Doublesided *labyrinthectomy*, bilateral severance of the eighth nerve, or paralysis of the labyrinths produced by injection of formalin through the round window markedly diminished the respiratory reaction. The elimination of the labyrinthine receptors, however, was not quite sufficient to abolish it. Extirpation of the labyrinths was, therefore, combined with transverse section of the *spinal cord*. In some experiments the operation on the spinal cord preceded, in others it succeeded the labyrinthectomy. It was, of course, necessary to perform the severance of the cord below the origin of the phrenic nerve. Usually the level of the last cervical segment or between this segment and the first thoracic segment was chosen. Such an operation by itself already reduced the respiratory reaction upon the vertical movements, while control stimuli, e.g., from the skin of the face

proved that the respiratory centers and tracts still reacted. After additional bilateral labyrinthectomy, the respiratory reactions on vertical

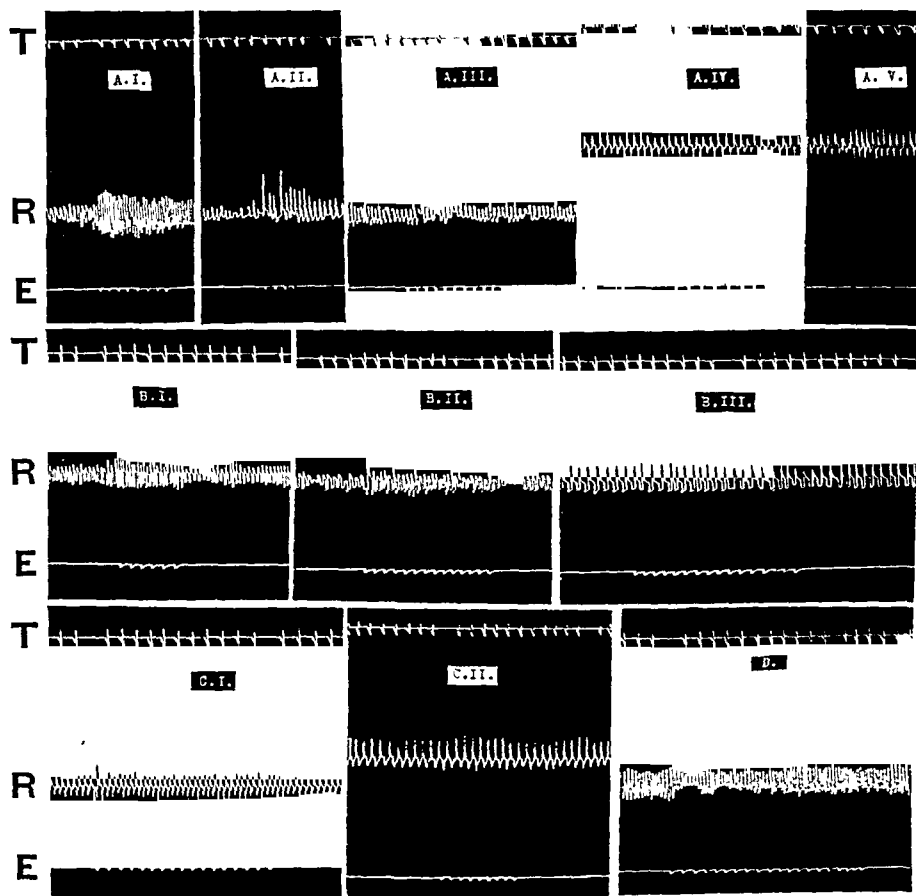


Fig. 1. Decerebrate and decorticate cats. A. Decerebrate cat. A.I. Respiratory reaction upon vertical movements. A.II. After transverse section between cervical and thoracic segments. A.III. Immediately. A.IV. Twenty minutes after formalin injection into both labyrinths. A.V. Control: reaction upon pinching the left foreleg. B.I. After extirpation of the cerebral hemispheres and the optic thalami; hypothalamus preserved. B.II. Hypothalamus extirpated. B.III. After transverse section behind the midbrain. C. Transverse section of the brainstem cranially to the midbrain. C.I. Respiratory reaction after transverse section of the first thoracic segment and severance of the cervical plexus. C.II. Respiration after formalin-paralysis of both labyrinths. D. Severe hemorrhage after decerebration. Inhibitory reaction.

R = respiration from trachea (expiration upwards). E = elevator movements (purely vertical movements). T = 5 second intervals.

movements were in some decerebrate animals no longer demonstrable (fig. 1, A). In some experiments traces of the reaction were still noticed. When the transverse section of the cord between the cervical and the

thoracic segments was in a decerebrate cat combined with severance of the cervical plexus (except the phrenic nerve), then subsequent labyrinthectomy completely abolished the respiratory reaction upon vertical movements (fig. 1, C). Control experiments showed that the respiratory center still reacted upon stimuli, e.g., from the area of the trigeminal nerve. As to the spinal tracts that carry the stimulating impulses to the respiratory center, it was found that bilateral labyrinthectomy plus severance of the posterior column and of the dorsal spinocerebellar tracts in the first cervical segment diminished but did not completely abolish the respiratory reaction upon vertical movements in decerebrate cats. This indicated that ascending systems in the lateral columns of the cord sending collaterals to the respiratory center (ventral spinocerebellar, or spinothalamic tract) may also cooperate in the reaction.

The inference may be drawn from all these experiments that impulses from the labyrinth, from the retina and finally impulses reaching the central nervous system with the spinal nerves (probably kinesthetic impulses from the skin and muscles) participate in producing the respiratory reactions upon vertical movements. Among these three groups, the impulses from the labyrinth and from the areas of the spinal nerves play (at least in the animals under study) a much more important part than the retinal impulses.

Relation to other vegetative labyrinth reflexes. It seemed necessary to analyze whether the labyrinthine impulses act primarily upon the respiratory center, or whether the reaction of this center is only secondary to labyrinthine reflexes upon other parts of the vegetative nervous system. Since stimulation of the labyrinth by rotation induces reflexly a fall of blood pressure (Spiegel and Démétriades, 1922), it seemed of interest to ascertain whether vertical movements have a similar effect upon the vascular system, and whether such a vasomotor reflex plays a part in the genesis of the above-mentioned respiratory changes. The blood pressure was recorded from the carotid or from the femoral artery by a mercury manometer. Since the manometer did not participate in the vertical movements of the animal, the changes of the animal's position in the vertical plane in relation to the manometer induced by themselves differences in the position of the recording lever. Various control experiments were, therefore, necessary. On dead animals, the carotid artery was connected with the manometer in exactly the same way as in the actual experiments. In controls on living animals the blood pressure was recorded with the animal held in the highest as well as in the lowest position of the elevator. A comparison of these control experiments with the records of blood pressure during the elevator movements gave no evidence that such an extent of vertical movements as produced in these experiments induces reflexly a change of blood pressure similar to the depressor effect of rotation. This

is particularly valid in pure rectilinear movements in the vertical plane. In the pitching movements sometimes a fall of blood pressure was noticed, particularly in the beginning of the movements or after they were stopped. Simultaneous records of the respiration showed that these changes were associated with single deep inspirations. In other cases, however, the blood pressure rose in the beginning of the pitching movements, while the change in respiration was the same as usual. The inference may be drawn that the changes in respiration brought about by vertical movements are not the consequence of reflexes upon the vasomotor center and of impaired blood supply of the respiratory center. They are also not caused by reflexes upon the digestive tract, as they appear also in animals that show no disturbances in this system. This indicates that impulses induced by vertical movements act primarily upon the respiratory centers.

SUMMARY

1. The influence of rhythmical rectilinear vertical movements and of pitching movements upon respiration was studied in dogs and cats.

2. Both types of movements induced an increase in frequency and amplitude of respiration. The effect is often most marked in the beginning of the movements, sometimes slow waves of increase and decrease in amplitude appear. The effect may outlast the stimulation, or may be followed by a period of inhibition (diminished amplitude of the respiratory movements).

3. In superficial anesthesia first the increase in the rate of respiration is affected, while the increase in amplitude during the vertical movements is still present. Deep anesthesia completely abolishes the reaction.

4. After extirpation of the cerebral hemispheres, the thalami, and the hypothalamus, the various types of the reaction could still be observed; if the reactivity of the respiratory center was depressed, sometimes an inhibitory reaction appeared. It is concluded that the respiratory reactions on vertical movements are at least partly due to brain stem reflexes.

5. An analysis of the importance of the various receptors in this reaction by extirpation experiments showed that mainly impulses from the labyrinth and from the area of the spinal nerves participate in producing the respiratory reactions upon vertical movements, while retinal impulses play only an accessory part.

6. These respiratory reactions appear independently of reactions of the vasomotor and digestive apparatus.

REFERENCES

- GRAHAM BROWN, T. *Pflüger's Arch.* 130: 193, 1909.
 POZERSKI, E. *Compt. Rend. Soc. de Biol.* 85: 702, 1921.
 SRÖBERG, A. A. *Acta otolaryngologica. Supplementum XIV. Exper. Studien ueber d. Ausloesungsmechanismus der Seekrankheit.* Stockholm, 1931.
 SPIEGEL, E. A. AND T. DÉMÉTRIADES. *Pflüger's Arch.* 196: 185, 1922. *Monatschr. f. Ohrenheilk.* 58: 1, 1924.

TEMPORAL SUMMATION IN PERIPHERAL NERVE FIBERS¹

E. A. BLAIR AND JOSEPH ERLANGER

*From the Physiological Department, Washington University School of Medicine,
Saint Louis*

Received for publication June 19, 1936

The phenomenon of temporal summation of subliminal afferent volleys to produce a reflex via the axial nervous system has long been known, but we have been unable to find any description of a comparable phenomenon in an axis cylinder. That temporal summation of impulses may occur in depressed peripheral nerve became apparent during the course of our observations on single axons, and this paper records our investigation of the phenomenon.

METHODS. The apparatus employed in general has been that used in the study of single axon spike potentials (Blair and Erlanger, 1933). The RCA 907 cathode ray tube now in use has proved sufficiently actinic to permit of the photographing of single sweeps across the face of the tube. The photographs, taken on 35 mm. motion picture film, are much sharper than the larger contact prints heretofore made by applying a film to the face of the tube. In all experiments on single axons the isolated phalangeal preparation of *Rana pipiens* has been employed. Usually by stimulation of one of the trunks of the sciatic plexus a preparation may be secured in which only one fiber conducts from the stimulated point to the lead electrodes on the distal attenuated portion of the nerve. The activity of this single fiber is observed by recording its amplified action potential. The rate of rotation of the interrupter synchronizing sweep and stimulation has been approximately 25 per minute. The first of the paired stimuli employed (designated S_1) has been the break shock from a Porter induction coil, the second (S_2), the discharge of a condenser through a grid-controlled, gaseous discharge tube (RCA 885) activated by the break of the primary current acting through the usual variable delay circuit. The RC product of the condenser discharge circuit has usually been of the order of 7.5×10^{-5} . Nonpolarizable Hg-calomel electrodes have been used exclusively. The potential difference between adjacent electrodes has rarely exceeded 3 millivolts.

RESULTS. *Initial observation of summation through accidental depression.* It frequently happens during observations on single axons, if and as con-

¹ This investigation was aided by a grant from the Rockefeller Foundation to Washington University for research in science.

ductivity fails, that a stage is reached during which a single adequate stimulus at a remote point never elicits a response at the lead, whereas a shock delivered after the first, and at the same point, results in a spike that is conducted the whole way, but only, of course, when it follows the first. If S_1 is weakened, at a critical level the response to S_2 disappears, but returns when S_1 is increased to its original strength. If the two shocks are approximated the response at the lead disappears at a critical interval; when separated beyond this value, it reappears. Two adequate shocks consistently result in a response at the lead when a single shock, even when increased many times above threshold, never causes a spike to appear. Apparently S_1 is producing a response which does not reach the lead, but modifies the nerve so that a second response, initiated in proper relation to the first, is conducted through.

This conclusion has been substantiated by a chance observation on an axon which gave a normal spike at both lead electrodes, but was obviously depressed in the interelectrode region: the response to S_1 was conducted to the electrode proximal with respect to the stimulus, but was blocked in the interelectrode region so that the spike was monophasic. Under these conditions the response to S_2 , following S_1 within a wide time range, was invariably conducted through the depressed region to the other electrode, as indicated by the diphasicity of the response. When S_1 was adjusted to threshold so that the fiber was stimulated in about half the trials (see Blair and Erlanger, 1933), and S_2 kept at a superthreshold level, whenever there was a response to S_1 , the response to S_2 would reach the distal electrode and produce a diphasic spike; whenever S_1 failed to stimulate, the response to S_2 would be blocked before reaching the distal electrode, and the spike would be monophasic and identical with that produced by S_1 . Since the experimental conditions remained constant in this experiment, the shock escapes can be eliminated as a possible factor, and the passage of the second impulse can be referred to a change in the depressed region resulting from the preceding impulse which reached, but did not pass through, the interelectrode region.

Summation through experimental depression. Having established the fact that there are chance conditions under which a single impulse will not be conducted the full length of a fiber while the second of two impulses will, if initiated within an adequate interval, the attempt was made to find a means of producing a similar condition experimentally in normal nerve. First the effect was determined of passing a constant current through a localized region between the stimulus and the lead. By careful adjustment of the intensity of the current the phenomenon was elicitable in every experiment of this type.

The question then arises, does the summation develop in the catelectrotonic or in the anelectrotonic region? To determine this, the nerve, to

describe a typical experiment, is continuously polarized through the lead electrodes by means of a circuit that has been described elsewhere (fig. 1, Erlanger and Blair, 1934). The lead and polarizing circuits are identical, so that it is possible to follow the development of anodal polarization at one electrode, and of cathodal polarization at the other. Since block occurs at the anode with weaker currents than at the cathode, the lead electrode proximal with respect to the stimulus is made anode. When the current is increased the amplitude of the spike recorded from the anode increases and the positive phase of the spike recorded from the cathode decreases in amplitude until a critical current strength is reached, when, due to block at the anode, there is a sudden decrease in the height of the negative phase of the spike and the spike becomes monophasic (see Erlanger and Blair, 1934). In figure 1 the spike, R_2 of $A1$ (produced by S_2 alone), has been reduced in height and made monophasic by this procedure. When, under these conditions, the fiber is stimulated earlier by S_1 , as in $A2$, the early response, R_1 , likewise is monophasic and low, but the second response, R_2 , is higher and diphasic; the latter now is being conducted to the distal lead. The interval between the shock artifact, S_2 , and the spike, R_2 , represents the conduction time. The artifact of S_1 in all cases is off of the record. Occasionally, as in $A3$, both responses may be blocked at the node nearest the lead. That the same phenomenon appears at other adequate separations of S_1 and S_2 is shown in the comparable series, B .

When the polarizing current is further increased the spike amplitude falls, again abruptly (as in $C1$), due to the blocking of another segment. But, as before, when the nerve is conditioned by R_1 the response, R_2 , is increased (as in $C2$) by the entrance of a previously blocked segment (compare $B1$ and $C2$). Occasionally, however, when two segments are thus blocked conduction is completely restored by the conditioning response; an instance is seen in $C3$ (compare $C3$ with $B2$). The latter observation may be interpreted as indicating that the response of a segment affects not only the next segment beyond the block, but also the second segment removed. As this phenomenon has been observed in but one preparation, for the present the possibility of an extension of this effect further than one segment must be left open, though the evidence seems to favor it.

Some determinations have been made of the order of magnitude of anodal polarization required to demonstrate temporal summation in peripheral nerve. Briefly, the voltage is of the order of 1 to 2 rheobases, and in some cases, with a small nerve, temporal summation has occurred with a current of less than 0.1 microampere. The usual current ranges between 0.7 and 2 milliamperes per square centimeter cross-section of nerve. The impression has been gained that if the rheobase is low the nerve will show temporal summation at or near rheobase; that if the rheobase is high voltages up to

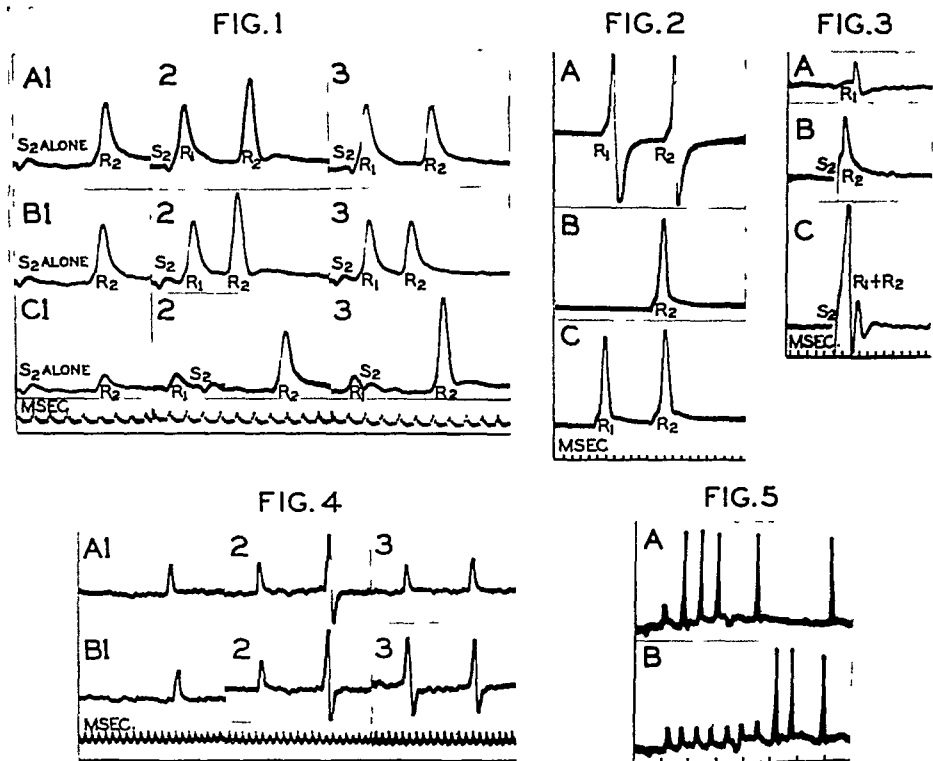


Fig. 1. Temporal summation in a fiber blocked by a current through the lead electrodes, anode proximal to the stimulus. The records are from the same single axon. 5/21/36. All figures are reproduced unretouched and in photographed size.

A. 1. The normal diphasic spike converted to a lower monophasic spike, R_2 , by anodal block. The spike is conducted to, but not past, the proximal lead.

2. Reestablishment of the higher, diphasic response, R_2 , by an antecedent, blocked response, R_1 .

3. An infrequent record showing block of both spikes, R_1 and R_2 .

B. 1, 2 and 3. As above, except for a shorter interval between the two spikes.

C. 1. Polarization increased so as to block an additional segment.

2. Reestablishment of this segment response by temporal summation.

3. An infrequent result showing reestablishment of conduction to the distal electrode by temporal summation through the two blocked segments.

Fig. 2. Temporal summation with multifiber spikes. 5/20/36.

A. Response (R_1 and R_2) of unpolarized nerve to two stimuli.

B. The spike, R_2 , made monophasic and lower by anodal block at the proximal lead.

C. The height of response, R_2 , increased by the antecedent response R_1 (Cf. R_2 of B).

Fig. 3. Records (5/14/36) showing summation of the effect on a blocked locus of an impinging response.

A. The nerve is stimulated by S_1 at a remote point, and the multifiber spike (about 12 fibers) reduced by anodal block.

B. Nerve stimulated by S_2 at the point of block so that only a few fibers respond.

C. Result of stimulation by S_2 at the time the spike from S_1 reaches the blocked locus.

2 rheobases may be required. If currents of this magnitude do not obtain physiologically, they probably are produced in the usual experimental preparation by demarcation potentials from sectioned fibers, and could very well be the cause of some, at least, of the incidental blocks that are encountered from time to time.

When the proximal electrode is made the cathode and the current increased while observing paired responses separated by about 15 msec., a stage is reached during which the first response of the pair is conducted through, while the second varies between full (cathodally polarized) height and a consistent, lower height. Apparently this stepdown in the height of the second spike is due to the failure of a previously conducting segment to respond. If the interval between the stimuli is reduced the amplitude of the second response falls off in steps in a manner entirely comparable to the quantal blocking resulting from increasing polarization (Erlanger and Blair, 1934). Manifestly in catelectrotonic nerve an effect develops that is the converse of temporal summation: a response makes the fiber less able to conduct a succeeding response.

The mechanism of temporal summation. Using anodal polarization as a means of producing a block which may be overcome by temporal summation of impulses, an effort was made to discover the underlying mechanism of this phenomenon. First, we endeavored to ascertain the shape of the curve of the temporal summation. Since summation is tested by a second response which, because of its all-or-none character, is not subject to independent experimental modification, the usual technique cannot be employed of expressing the effect of a conditioning stimulus in terms of the threshold of a testing stimulus. Only two points on the curve can be determined, namely, the minimum and the maximum separation of the two stimuli which will cause the second response to traverse the depressed locus. An effort was made to plot the curve indirectly by determining

Fig. 4. Temporal summation in a single axon depressed at the proximal lead by calcium. 5/29/36.

- A. 1. Calcium block converts a diphasic response to a lower, monophasic response.
2. An antecedent uncondacted response now results in propagation of a second response to the distal lead.
3. Record when both responses are blocked.
- B. 1 and 2. After block of both responses by calcium; temporal summation reestablished by a current of $0.02\mu\text{A}$ with cathode at the poisoned region.
3. Calcium action antagonized by a further increase in cathodal polarization so that both responses are conducted.

Fig. 5. Temporal summation of several impulses. 5/19/36.

- A. Effect on a repetitive burst of slight anodal polarization at the proximal lead.
- B. Increasing the polarization increases the number of responses required to overcome the block. In this record 7 spikes reach the lead before conduction across it occurs. Time intervals, 5 msec.

these two values for a range of polarization strengths, but the technique did not yield quantitative information because the nerve continues to change under polarization and the rate of change appears to vary with the polarizing current.

It seemed possible that in a large nerve a number of fibers might exhibit temporal summation at a given polarization level, and, if so, that the curve might be determined approximately by the number of fibers, indicated by the amplitude of the multifiber spike, showing summation at several separations of the stimuli. With this in mind, to describe a given case, the two shocks, both maximal for alpha (see fig. 2A), were separated by about 7 msec. As polarization through the leads was increased with anode proximal the amplitude of the response from S_2 at first increased, due (a) to the anodal increase in the height of the contributing spikes and (b) to the cathodal reduction of the diphasic artifact, and then decreased as the individual fibers were blocked. When the amplitude has thus been reduced to about half of the maximum height (fig. 2B), the effect of an antecedent response was determined on the height of R_2 . There was (see C) an increase amounting approximately to 6 per cent. No significantly greater values were found at other separations. The smallness of the increase is a reflection of the narrowness of the range of polarization which will produce a state showing temporal summation; in a multifiber response relatively few fibers are in this state at any given polarization strength. Obviously, the experimental range is not sufficiently wide to permit of the determination of a satisfactory curve of temporal summation.

Correlation of a large number of observations on single fibers in different preparations subjected to varying degrees of polarization indicates that the summation effect attains an early maximum and then declines slowly. Frequently the second response will be conducted through the blocked area as soon as the untreated nerve will conduct. We have seen summation with a stimulation interval of only 0.8 msec., when the second spike, though delayed through conduction in relatively refractory nerve, occurs so early on the negative after-potential that it appears to terminate the first spike. It is possible, however, for the second impulse to arrive at the lead too early to be propagated; this has occasionally been seen, but in no case has the appearance of summation been delayed longer than 3 msec. Apparently the curve reaches a maximum at from 2 to 4 msec. The decline of the effect, as indicated by wide changes in maximum summation time with slight changes in polarizing current, is very gradual. That the decline is slow is indicated also by the fact that there is a wide time range between conduction of all second responses and failure of all, signifying that summation declines by an amount equal to the range of the spontaneous change in the excitability of the axon over a relatively long period (Blair

and Erlanger, 1936 a and b). Very frequently the period of summation has lasted in excess of 100 msec.

Since a second response is conducted through a depressed region when the first is not, the phenomenon might be attributable to 1, an increase in the stimulating ability of the last responding segment, or 2, an increase in the excitability of the segment which failed to conduct the first response.

1. If the action potential is not actually responsible for restimulation it, at least, seems justifiable to assume that it is proportional to the stimulating power of any responding segment. Granting this, it follows that if the stimulating power of the last conducting segment is increased by a preceding response, this should be manifested as a supernormality of spike amplitude. To ascertain whether or not spike height is increased a state may be produced, by suitable polarization strength, in which the first response always is blocked, while the second is conducted in a vast majority of the trials. If temporal summation is referable to supernormality of spike height, then in the infrequent instances of failure of the second response to be conducted, the spikes which just fail of conduction should be higher than the first spike which never causes a response of the blocked segments. Figure 1, $A\beta$ was secured under these conditions. R_1 never was conducted through, whereas R_2 failed infrequently. Certainly R_2 is no higher than R_1 . $B\beta$ was secured under the same conditions except for a shorter interval between stimuli. The second potential, even when the spike is added to the after-potential of the first, is definitely lower. Temporal summation, therefore, cannot be referred to an increase in the voltage of the response of the segment adjacent to the block due to a previous response, and inferentially temporal summation cannot be referred to an increase in the stimulus acting on the depressed locus.

2. Since the site of temporal summation apparently is not the last unblocked segment, the summation must be attributed to a change in the excitability of the blocked segment and should be demonstrable as a change in its electrical threshold. This deduction could be tested if a locus on a fiber could be blocked and the threshold determined there before and after the arrival of a spike which reached, but did not traverse, the locus. The experiment, however, is not feasible with the present preparation because the whole operation would have to be confined to a single trunk of the sciatic plexus in order to limit the response at the lead to a single fiber, and the trunk is not long enough for the purpose. Therefore the attack has been made by stimulating the whole sciatic nerve, instead of one of the trunks, and securing a record of the summed spikes of about twelve fibers, instead of but one. By polarizing through a pair of electrodes, with anode proximal to the lead and about 5 mm. from it, the spike is reduced by blocking all except two or three of the fibers responding to a shock (S_1) applied

at the central end of the nerve (fig. 3A). The proximal amplifier lead on the distal end of the nerve is then connected to record from the blocking electrode and the conduction time from S_1 to the depressed locus is determined. The amplifier is then reconnected to the original lead electrode and a shock (S_2) is adjusted to stimulate two or three fibers at the blocked locus (record B). When the two shocks, S_1 and S_2 , are simultaneous, the response to the latter is not modified, showing that there is no interaction of shocks. When S_2 is applied to the polarized region, say, 30 msec. after the nerve has been stimulated by S_1 , the presence of the first shock does not alter the response to S_2 . But if the interval between shocks is then reduced, at about 20 msec. the addition of the first shock causes additional fibers to respond to the second. Still more fibers respond to the second shock as it is brought closer to the first, the increase produced by the latter apparently reaching a maximum when the separation is equal to the previously determined conduction time from the upper stimulating electrodes to the depressed locus. In record C, the two shocks having this separation produce a much higher spike ($R_1 + R_2$) than the sum of the two responses of A and B when the shocks are delivered separately, indicating the stimulation of additional fibers. When the second shock is delivered after an interval that is less than the conduction time, its spike precedes that from the earlier, more distant, stimulus and is not modified by it. The experiment demonstrates that a fiber response which is not conducted through a depressed segment, nevertheless may lower the threshold of the segment to electrical stimulation, and, inferentially, to a second propagated spike. In addition it can be taken to demonstrate that the curve of summation of shock and spike at a depressed locus is at a maximum approximately at the time of arrival of the spike, and then decreases at a very slow rate. It may not be superfluous to add that the total period of lowered threshold greatly outlasts the duration of the spike.

If the lowering of the threshold of a depressed segment produced by a spike impinging on it is maximal approximately coincidental with its arrival, one would expect the same relation to hold when dealing with temporally separated spikes. But, as mentioned above, the greatest temporal summation appears to occur with an interval between two spikes of from 2 to 4 msec. This difference apparently is to be referred to the fact that the refractory phase of the last conducting segment lasts some 2 to 4 msec., whereas the increased excitability of the blocked segment seems to fall very slowly; and an early, and consequently low, second spike in the last conducting segment may not stimulate beyond although the excitability of the blocked segment then is highest, whereas a later spike of normal amplitude will stimulate the still, though less, hyperexcitable segment.

Temporal summation through other than electrical blocks. All of the

observations on temporal summation that have been considered up to this point could be simply accounted for as being due to the effect of the negative action potential in opposing the polarizing current producing the block, the reduction of the induced depression then permitting a second response to be conducted. In order to ascertain whether this is a necessary combination other blocking agents, not involving external currents, have been employed. Ether, a commonly used blocking agent, produced a block with the same general characteristics as that elicited by cathodal polarization. From the point of view of behavior of the blocked region etherized and catelectrotonic nerve are indistinguishable.

Calcium, chosen because of the similarity of its action to anodal polarization, produced a block which showed temporal summation identical with that demonstrable by anodal polarization. In these experiments 1 per cent calcium chloride was jetted into the proximal lead electrode (see Erlanger and Blair, 1934). As diffusion raised the calcium concentration in the nerve the nerve response varied in a manner paralleling the variation produced by increasing anodal polarization, although, as indicated by the absence of current in the usual polarizing circuit, the electrodes remained essentially isoelectric. Figure 4, *A* is a series of records, comparable with those of figure 1, *A* and *B*, but showing temporal summation through a calcium block. A concentration of calcium below that required to block elevates the rheobase and reduces the amount of current necessary for block, apparently adding its effect to that of the anodal blocking current; and it subtracts from the cathodal rheobasic current. After the concentration of calcium had increased to the point of blocking both of the paired responses, the nerve was polarized cathodally at the depressed locus by 0.02 microampere and, as shown in *B1* and *2* of figure 4, temporal summation again could be demonstrated. That calcium acts in a manner analogous to anodal polarization is further shown by the fact that if cathodal polarization is increased, a calcium depression can be overcome so that both the first and the second responses will be conducted (see *B3*).

Figure 4 shows in *AS* the only case of supernormality of spike height observed in the whole series of experiments with polarization and with calcium. Anodal polarization and calcium both produce blocks which can be overcome by temporal summation without supernormality of spike height, but the present case indicates that under certain conditions with calcium, and possibly with other agents, increased response of the unblocked segment may play a part.

Temporal summation with repeated conditioning responses. Since the decreased threshold resulting from a spike impinging on a blocked segment far outlasts the refractory phase, it should be possible to sum the effect of several uncondacted spikes to finally produce propagation through a block. An opportunity to study this phenomenon was presented by a fresh

preparation in which a single shock caused a repetitive response of the single axon conducting to the lead. By adjusting the strength of a blocking current with anode at the lead the picture could be progressively changed from the usual result of a single conditioning response blocked at the lead causing conduction of subsequent responses (fig. 5A) to one in which the effect of seven conditioning responses summed to eventually produce conduction (5B). A similar summation of the effect of blocked spikes is shown in a figure previously published (fig. 3, Erlanger and Blair, 1936). There the first few monophasic responses serve to condition a depressed locus between the leads so that the spike is permitted to pass, as indicated by the subsequent diphasic responses. The occasional monophasic responses occurring in that figure after a diphasic series has been started is referable, presumably, to a long refractory phase in the depressed locus.

DISCUSSION. The evidence that the reactivity of a blocking segment can be raised by a blocked impulse seems to be indubitable; it is possible, even, that two adjoining segments can be thus affected. Taken at their face value, these observations imply that the last responding segment exerts an influence that radiates beyond the segment's limits a distance spanned by one or possibly two segments, roughly 1 or 2 mm. Electricity seems to be the only adequate agency that could radiate that distance in the time available. Our observations, therefore, signify that propagation in nerve is accomplished by a mechanism that is in part, at least, electrical. It does not seem possible to attribute the summation to a mechanism involving the liberation of neurohumors.

Temporal summation commonly is supposed to be an attribute of synaptic junctions in the nervous system, and synaptic junctions are characterized primarily by the discontinuity of the connection between the contiguous elements. This supposition is supported by the fact that an artificial discontinuity or block along the course of a fiber provides the means of demonstrating temporal summation.

It was stated above that we have not succeeded in finding in the literature any evidence for temporal summation in nerve fibers. There has been described, however, a phenomenon which simulates it. Bethe and others (see Schaefer and Schmitz, 1933, for the literature), using a nerve-muscle preparation, have found that local compression of the nerve at an intermediate point increases the height of the contractions of the muscle elicited by stimulation of the nerve with induction shocks. Now, one might conceivably maintain that in this experiment the compression serves as a second stimulus which sums temporally with the effect produced by the shock. Schaefer and Schmitz show, however, that the multifiber spike of a nerve actually is lowered by passing it through a compressed locus. Of significance in this connection is the evidence they present indicating that

injured fibers may continue to discharge for a time after conducting an impulse. Presumably the fibers under these circumstances respond with a brief repetitive burst (see Erlanger and Blair, 1936) and the increased height of the contraction in experiments with a nerve-muscle preparation, therefore, is to be attributed, not to temporal summation in the nerve, but to summation of contractions in the muscle,—to the conversion of a twitch into a brief tetanus.

SUMMARY

1. A state in a single axis cylinder such that a single impulse will not be conducted, but impulses succeeding within about 100 msec. will, can be produced by anodal polarization and by calcium poisoning, and also occurs fortuitously in failing nerve.

2. This temporal summation is referred to a change in the excitability of the blocked segment induced by the first response, which, though blocked, lowers the threshold beyond so that the next response, which may be lower, and typically is no higher, than the first, reaches the threshold of, and is conducted through, the depressed area.

3. Cathodal polarization and ether produce a state in nerve such that the second of two responses will not be conducted when the first is. By approximating the two stimuli under these circumstances it is possible to decrease the second spike in steps in a manner entirely comparable to the serial blocking of segments induced by increasing the intensity of depressing agents.

We are indebted to Dr. Hubert Peugnet for assistance in the designing of our present electron oscillograph plant. Doctor Peugnet collaborated with us during the initial observations of this investigation.

REFERENCES

- BLAIR, E. A. AND J. ERLANGER. This Journal 106: 524, 1933.
This Journal 114: 309, 1936a.
This Journal 114: 317, 1936b.
ERLANGER, J. AND E. A. BLAIR. This Journal 110: 287, 1934.
This Journal 114: 328, 1936.
SCHAEFER, H. AND W. SCHMITZ. Ztschr. f. Sinnesphysiol. 64: 161, 1933.

CONTROL OF URINE FORMATION IN THE FROG BY THE RENAL CIRCULATION

EDWARD F. ADOLPH

From the Department of Physiology, School of Medicine and Dentistry, The University of Rochester, Rochester, N. Y.

Received for publication March 17, 1936

Do changes in the blood's circulation within the kidneys account for changes in the rates of urine formation (water excretion)? Ludwig (1844) suggested that such was the case. The opportunity to investigate the question for the frog arose because the circulation of blood through glomeruli could often be seen to change when water excretion stopped (Adolph, 1934; 1935a). Further observations showed that when the glomeruli were not visibly affected, other circulatory modifications could be identified.

Previous studies of glomerular circulation in amphibia were made, beginning with the work of Richards and Schmidt (1924) and Hill and McQueen (1921). In their studies, however, the rates of urine formation were not measured, so that the circulatory factors could not be correlated with actual performance in the kidneys. The notion has become prevalent, nevertheless, that glomerular blood flow is an index to urine formation. Some attempts to correlate urine formation with blood flow were made by Tamura et al. (1927), but urine formation was not measured over short intervals of time, foreign fluids were introduced into the circulation, and decisive factors were not employed to modify rates of urine formation.

The data of Richards and Walker (1935) and others affirm that in the frog fluid is separated from the blood while it is in glomerular capillaries. In the courses of the tubules, water and other constituents escape from the lumina. Hence the chief site of the control of volume output might be either in Malpighian bodies or in tubules, or equally in both.

METHODS. Each frog (*Rana pipiens*) was operated upon by crushing the brain, opening the abdomen, and exposing one kidney for observation. In the chief experiments the ureter leading from this kidney was cannulated, and while urine flowed into the cannula the position of the meniscus was read at one-minute intervals. In other experiments blood pressures were measured by connecting one cannulated aorta to a mercury manometer. In the kidney, selected glomeruli were observed by transmitted light, and presence or absence of blood flow in them was recorded. Heart rates were likewise observed. The entire frog was enclosed in a sealed

chamber and kept basally in an atmosphere of oxygen at atmospheric pressure, and usually at 20° to 22°C.

Changes in rate of urine production were induced by numerous agents. Agents that were in the gaseous state were administered by replacing the oxygen by another gas mixture. Agents that had to be applied intravenously or subcutaneously were injected from outside the chamber through very narrow rubber tubes. Agents applied to the skin were in solutions soaked up in cotton and placed on the skin by momentarily opening the chamber.

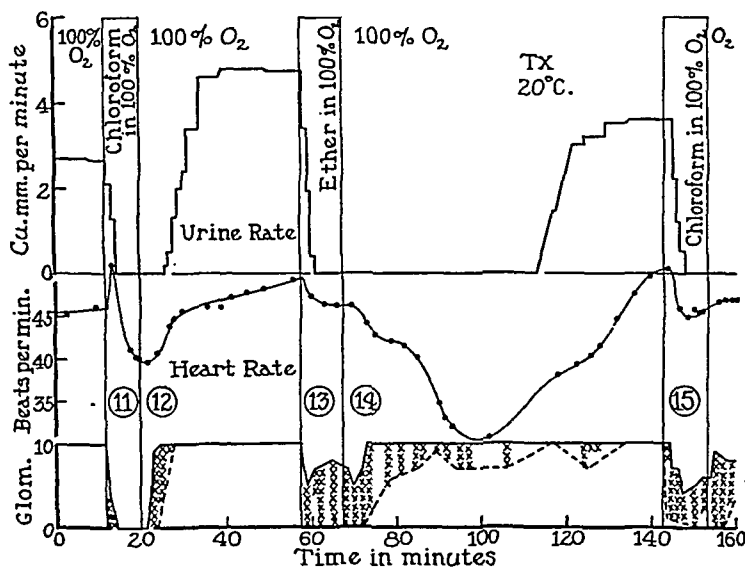


Fig. 1. Simultaneous rates of urine formation, pulse rate, and blood flow in glomeruli of frog *Tx*. The midbrain was crushed and the right ureter cannulated, and the animal was enclosed in a sealed chamber through which oxygen passed. The urine rates were measured by reading the meniscus in the ureter cannula at 1-minute intervals. In the right kidney ten glomeruli were watched; ordinates indicate the number of them through which blood flowed; the crosses indicate in which it flowed unusually slowly.

RESULTS. *Examples of the results.* In previous papers (1935a; 1935b; 1936) experiments were described in which simultaneously with cessation of urine formation all visible glomeruli ceased conducting blood. Both the circulatory changes and the changes in rate of water excretion occurred at the same moment, in response to administration of epinephrine or carbon dioxide, or to lack of oxygen. Upon the restoration of the basal conditions the circulation in the glomeruli was restored, and the urine formation resumed its previous control rate. Evidently the circulatory events were sufficient to account for the anuria, for it has been attested by many experiments that no urine forms when blood ceases to flow in all glomeruli.

Another type of experiment illustrating additional features is shown in figure 1; anesthetics were administered in the form of vapor while the atmosphere of oxygen was maintained. Urine rate, heart rate, and flow of blood in glomeruli changed almost instantaneously upon each administration of anesthetic. In the first administration (chloroform) it can be seen that the heart rate temporarily increased while the visible glomerular blood flow ceased entirely; hence it is evident that these two circulatory changes are not necessarily parallel. After the anesthetic was removed, the recovery of all three functions was prompt. At the second administration of anesthetic (ether) the blood did not stop flowing in all of the glomeruli, yet urine formation stopped completely. The recovery of glomerular activity was relatively prompt, but the heart rate now indicated grave

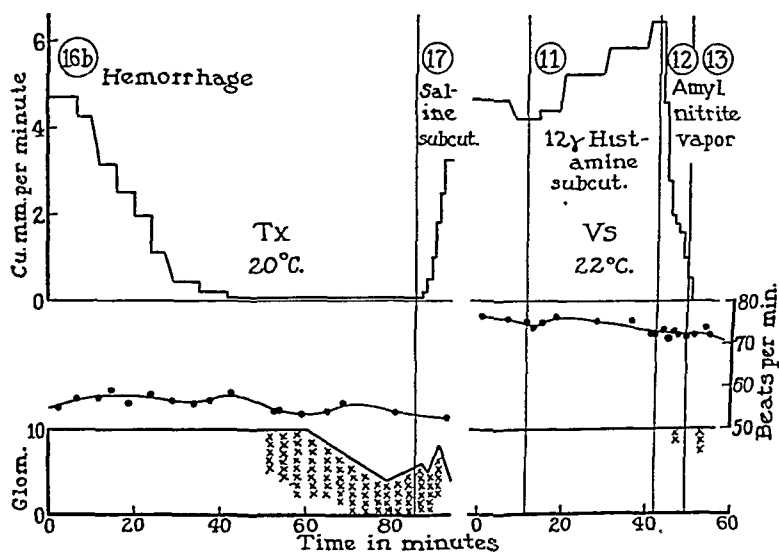


Fig. 2. Simultaneous rates represented in the same manner as in figure 1, in male frogs Tx and Vs, each weighing 26 grams and kept in an atmosphere of oxygen.

difficulty in the circulation; the systemic inadequacy coincided with the failure of urine formation for a period of 45 minutes. One notable feature was that the urine formation now became maximal before the heart rate reached its maximum. After the third administration of anesthetic the recovery of urine formation did not occur for a period of almost an hour, with prolonged but reversible slowing of the heart.

The experiments of figure 1 indicate the general facts which have been observed in numerous other experiments. These facts are: in order that urine formation shall proceed, blood must flow in at least some of the glomeruli and the general circulation must be in good condition. The converse does not hold, since even though the general circulation be excellent and some glomeruli be conducting blood, there may be no urine

formation. In other words, glomerular blood flow does not insure urine formation.

Other agents regularly produced oliguria or anuria without any specific effect upon the blood vessels leading to the glomeruli. An example is afforded by the hemorrhage experiment plotted in figure 2; the diminution of urine flow accompanied a reduction of glomerular blood flow and of heart rate. Recovery occurred very promptly when isotonic salt solution was injected into the lymph spaces. A similar change in the general circulation followed the administration of amyl nitrite vapor (fig. 2); the rate of heart beat also diminished rapidly in subsequent minutes.

Certain agents were found that produced oliguria without obviously modifying the rate of heart beat or the glomerular blood flow; examples

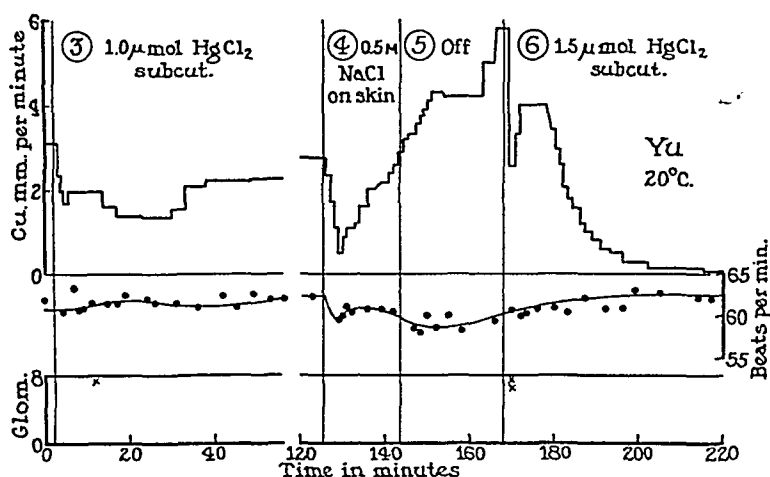


Fig. 3. Simultaneous rates represented in the same manner as in figure 1, in male frog *Yu* weighing 38 grams and kept in an atmosphere of oxygen. The two oliguric agents tested here produced no significant changes in heart rate and only momentarily in blood flow through glomeruli.

are shown in figure 3. Mercuric chloride, injected into lymph spaces in two different doses, reduced the urine output to 45 per cent of the basal rate and to zero; yet in neither case was any persisting reduction of the glomerular circulation manifested. A circulatory change in glomerular blood flow was regularly exhibited in the first 2 or 3 minutes after administration; this coincided with the initial fleeting oliguria at the time of 170 minutes (fig. 3). Occasionally renal arterioles constricted momentarily in response to almost any change of environment or administration of agent. Persisting circulatory changes occurred after mercuric chloride injection, for the amplitude of heart beat decreased progressively. In two of five tests, the rates of urine formation returned to normal spontaneously, and in *Yu* 6 the injection of a moderate amount of saline was followed

by temporary but marked urine production, without debris appearing in the urine. Another agent that gave little observable indication of influence upon glomerular blood flow or heart rate was a strong solution of sodium chloride placed on the skin (fig. 3). Recovery from this oliguria was spontaneous. For further analysis of the effects of these agents the measurement of arterial blood pressure was required.

Control experiments were found in the cases of several agents that failed to influence urine formation to significant extents. Ethyl urethane, when applied to the skin for a sufficient length of time to abolish reflexes, and histamine, injected subcutaneously, had no effect; and neither of them produced visible change in the circulation. Another control procedure, injection of isotonic sodium chloride solution, was without oliguric effect, as previously reported (Adolph, 1936).

Classification of oligurias. The degrees to which the renal *blood flows* were modified when urine formation was reduced by the various agents may be compared by the following procedure. The number of glomeruli being watched that maintained a flow of blood within their capillaries in the control period was recorded at brief intervals during the experimental period. The proportion of glomeruli that ceased to conduct blood was thus ascertained; further those glomeruli which conducted blood obviously more slowly than normal were credited with an activity of half the normal. Thus in the absence of total closure of any one glomerulus the activity was not evaluated at less than 50 per cent. It must be understood that the "glomerular activities" recorded have not the quantitative basis that the other changes possess. The *heart rates* were compared by taking the control period just previous to represent a rate of 100 per cent. The *urine rates* were compared in the same way, taking the previous control period as 100 per cent. All the control periods were characterized by an atmosphere of pure oxygen about the non-breathing frog.

The results of correlating these three quantities are given in table 1. Careful consideration of the data on circulatory conditions, particularly those of glomerular activity, leads to division of the experiments into five categories, and the agents tested are tabulated in an order that corresponds. In group *A*, the effect of the agents producing oliguria was to reduce the blood flow through the glomeruli and to stop it completely in a great many of them; yet the general circulation and the heart rate improved. In group *B*, the effect was likewise to reduce specifically the blood flow in glomeruli, but at the same time to embarrass the circulation in the whole body, with decrease in rate of heart beat. In group *C*, the reduction of blood flow in the renal glomeruli was only part of a depression of the systemic circulation, which was, however, entirely reversible. In group *D*, no circulatory change of magnitude sufficient to slow visibly the flow of blood through the glomeruli was noted; when the arterial blood pressure

was measured, significant changes were revealed. In group *E*, there was no significant oliguria and no visible effect upon the circulation. It is noteworthy that no agent was found that conversely produced a circulatory change without affecting urine formation.

Time relations. Further analysis of the correlation of circulatory and renal changes in response to various agents was made by comparing the times required for the onset of oliguria and of recovery from it. The

TABLE 1

Mean ultimate rates of urine formation, pulse rate, and approximate glomerular activity, as percentage of those in control periods, shown during exposures to various oliguric agents by pithed frogs at 19° to 26°C.

AGENT	DOSE	NUMBER OF TESTS	NUMBER ANURIC	URINE RATE per cent	HEART RATE per cent	GLOMERULAR ACTIVITY per cent
A. 1. Epinephrine subcutaneously.	200-650 γ /kgm.	6	1	16	103	18
Epinephrine by vein.....	26-310 γ /kgm.	8	1	27	104	24
2. Infundin subcutaneously....	2-92 units/kgm.	6	5	3	104	0
Infundin by vein.....	0.8-1.6 units/kgm.	3	2	3	106	30
B. 3. Ethyl ether vapor.....		5	4	2	87	16
4. Chloroform vapor.....		3	3	0	85	15
5. Chloretone on skin.....	0.04 M	4	2	8	89	30
Chloretone by vein.....	84 μ mols/kgm.	2	0	45	92	60
6. Oxygen lack, a.....	0.2 per cent	11	11	0	72	0
C. Oxygen lack, b.....	10-13 per cent	7	2	8	79	90
Oxygen lack, c.....	21 per cent	5	0	25	94	100
7. Carbon dioxide.....	29-31 per cent	9	7	2	95	70
8. Amyl nitrite vapor.....		3	2	4	86	60
9. Hemorrhage.....		2	1	10	91	32
D. 10. Na oxalate subcutaneously..	350-600 μ mols/kgm.	3	1	30	96	83
11. HgCl ₂ subcutaneously.....	26-58 μ mols/kgm.	5	3	10	104	79
12. Concentrated NaCl on skin..	0.5 M	3	0	35	99	96
13. Drying the skin.....		2	0	40	99	100
E. 14. Urethane on skin.....	1.1 M	3	0	76	98	100
15. Histamine subcutaneously..	63-630 γ /kgm.	3	0	128	102	100
16. 0.1 M NaCl subcutaneously.	5-22 cc./kgm.	4	0	156	104	100

results of such comparison are indicated in table 2. At the onset of oliguria, either the glomerular activity or the cardiac activity was obviously diminished simultaneously or within 1 minute of the first diminution of urine rate. This held for the individual experiments, as well as for the averages of many experiments.

In the recovery period the glomerular activity was restored rather promptly; the pulse rate either equally promptly, or, in the case of the

volatile anesthetics, with some delay. The urine rate often recovered much more slowly than the circulatory factors. Evidently the circulatory factors were prerequisite for urine formation, but adequacy of pulse rate and glomerular blood flow did not always insure urine formation. Some other changes which might recover more slowly than either of the measured circulatory changes observed appeared to be required in many instances.

Arterial blood pressures. In what circumstances are changes of heart

TABLE 2

Mean latent periods, in minutes, of earliest visible renal and circulatory changes simultaneously observed in the experiments listed in table 1

All rates decreased at onset and increased at recovery unless otherwise (†) noted. Some recovery times (*) are measured from the time of application of the agent instead of from its removal.

AGENT	ONSET OF OLIGURIA			START OF RECOVERY		
	Urine	Heart	Glo-meruli	Urine	Heart	Glo-meruli
A. 1. Epinephrine subcutaneously.....	2	3†	2	10		5*
Epinephrine by vein.....	1	2†	1	5		4*
2. Infundin subcutaneously.....	2	2†	2	38		20*
Infundin by vein.....	1	2†	2	16		10*
B. 3. Ethyl ether vapor.....	1	2	1	20	8	3
4. Chloroform vapor.....	2	2	1	22	7	2
5. Chloretone on skin.....	1	1	1	21		16
Chloretone by vein.....	1	2	2	5		*
6. Oxygen lack 0.2 per cent.....	2	3	2	7	1	2
C. Oxygen lack 10-13 per cent.....	5	6	6	4	1	2
Oxygen lack 21 per cent.....	6	7	8	6	2	10
7. Carbon dioxide 29-31 per cent.....	3	4	7	10	3	4
8. Amyl nitrite vapor.....	1	2	3	14	2	
9. Hemorrhage.....	2	5	3	40		*
D. 10. Na oxalate subcutaneously.....	7	7	12	58		46*
11. HgCl ₂ subcutaneously.....	1	2†	1	30		*
12. Concentrated NaCl on skin.....	2			6		*
13. Drying the skin.....	6			2		
E. 14. Urethane on skin.....	1			7		*
15. Histamine subcutaneously.....	6†					
16. 0.1 M NaCl subcutaneously.....	1†	2		12		*

rate indicative of changes of blood pressure? Arterial pressures were measured in the aortic arches of frogs that were otherwise treated in the same ways as when rates of urine formation had been measured.

The results of the study of arterial pressures are shown in table 3. Here are included data of certain other investigators obtained under conditions that appeared to correspond closely to those prevailing in the present measurements of rate of urine formation. The most striking feature is

that the changes of arterial pressures correlate accurately with the changes of urine rates for agents 3 to 16 inclusive. The latent periods for the be-

TABLE 3

Mean ultimate aortic blood pressures and heart rates (as percentage of those in control periods), and latent periods, shown by operated frogs in brief exposures to various oliguric agents at 18° to 22°C.

* Frogs not operated; † Toads

AGENT	DOSE	NUMBER OF TESTS	AORTIC PRESSURE	LATENCY OF ONSET	ULTIMATE EFFECT AT	HEART RATE
			per cent	min-utes	min-utes	per cent
A. 1. Epinephrine subcutaneously.....	5 γ 200-2500 γ /kgm.	8	145 C		8	
Epinephrine by vein..		7	133 K	1		
2. Infundin subcutaneously.....	3.7 units/kgm.	2	106 N	1	8	103
Infundin by vein....						
B. 3. Ethyl ether vapor...	0.04 M	3*	64 B			
4. Chloroform vapor...		2	79 N	1	5	84
5. Chloretone on skin..		2	82 N	4	11	93
Chloretone by vein..						
6. Oxygen lack, a.....	0.2 per cent	8	64 A ₁	2	8	75
C. Oxygen lack, b.....	10-13 per cent	2	89 A ₂	5	11	95
Oxygen lack, c.....	21 per cent	4	82 N	5	21	96
7. Carbon dioxide.....	29-31 per cent	3	75 N	5	17	84
8. Amyl nitrite vapor..						
9. Hemorrhage.....		9†	72 H		3	95
D. 10. Na oxalate subcutaneously.....	250-400 μ mols/kgm.	3	81 N	5	15	101
11. HgCl ₂ subcutaneously.....	19-37 μ mols/kgm.	6	77 N	2	19	102
12. Concentrated NaCl on skin.....	0.5 M	3	93 N	2	6	98
13. Drying the skin....						
E. 14. Urethane on skin....	1.1 M	1	107 N	1	4	101
15. Histamine subcutaneously.....		Few	100 C			
16. 0.1 M NaCl subcutaneously.....	5-12 cc./kgm.	3	133 N	3	11	102

A₁ = Adolph, 1934; A₂ = Adolph, 1935b; B = Bieter and Scott, 1929; C = Cullis and Scarborough, 1932; H = Hofmeister, 1889; K = Kuno, 1914; N = new.

ginnings of pressure changes are in agreement with the latent periods for changes of heart rate shown in table 2.

The statement may be made that the rates of heart beat are indications

of the blood pressure changes produced by agents of groups *B*, *C*, and *E*, but are not so for group *D*. Evidently agents of group *D* diminish the systemic arterial pressures but do not affect the heart rates unfavorably.

Pressure responses to agents 10, 11, and 12 (group *D*) are portrayed in figure 4. Evidently marked decreases of pressure occurred in the first two, even though the heart rates remained constant or else increased slightly. To the third agent (0.5 M NaCl on the skin) only a temporary decrease was shown; but this corresponds exactly to the course of the oliguria occurring (fig. 3). As may be noted in figure 4, the arterial pressures, after decreasing, frequently failed to return to the original levels. Yet it is known that urine flow usually recovered promptly, and this may point to the occurrence of local adjustments in renal blood-vessels. Dry-

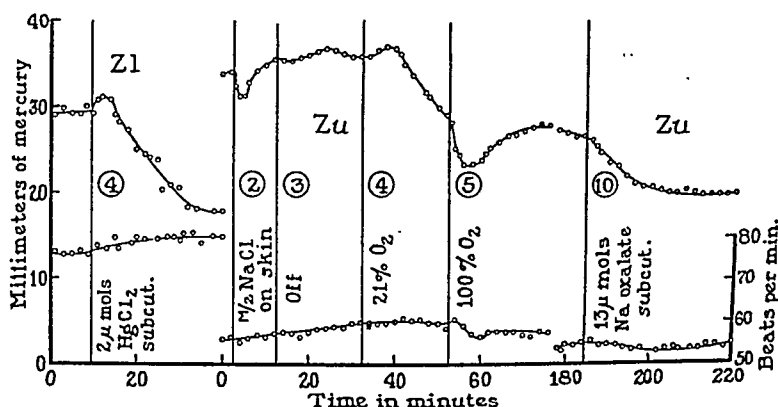


Fig. 4. Pressures in the left branch of the aorta, and simultaneous rates of heart beat. Frog *Zl* of 54 grams and frog *Zu* of 50 grams, kept in an atmosphere of oxygen. The character of the pressure changes may be compared with the urinary rate changes shown in *Yu* (fig. 3) in which similar doses of some of the same agents were administered.

ing the skin (agent 13) always involved some exposure of the frog to cooling by evaporation. Schulz (1906) found that a lowering of temperature from 18° to 8°C. decreased the arterial pressure to 79 per cent and the pulse rate to 30 per cent. It is concluded as the result of much study, however, that cooling is not the only factor involved in the oliguria thus produced; yet in any measurement of blood pressure this sequel of cooling will manifest itself while the skin is drying, masking other factors.

Measurements of aortic pressure (agent 6c) in the presence of room air (21 per cent oxygen) now showed distinct decreases (fig. 4) compared to those in the presence of 100 per cent oxygen. The fact was previously reported (Adolph, 1935b) that urine production of the non-breathing frog was slow in room air.

The times at which pressure effects occurred in *renal* vessels were also

measured. Changes in what may be referred to as "afferent arteriolar pressure" were ascertained by successively introducing fluid under pressure through the ureter into the urinary passages during 10 to 15 seconds, and noting at each imposition of pressure whether blood ceased to flow into the capillaries of certain glomeruli. It was ascertained that no further changes of flow occurred after 10 seconds of pressure. If the flow into one glomerulus stopped and not into another, this was taken to indicate the mean systolic pressure at the points of entrance of blood into the glomeruli. By this procedure it could be found whether pressures fell in the renal arterioles under the same circumstances as in the aorta.

An experiment is shown in figure 5. It was found that the local arteriolar pressure changed with the same latencies as the rate of heart beat.

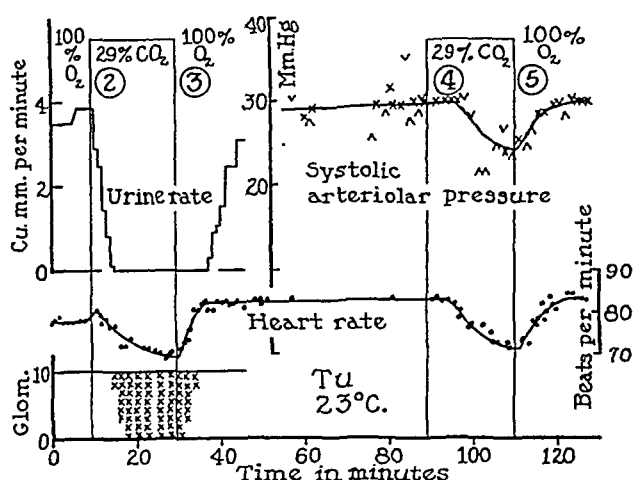


Fig. 5. Data for rate of urine formation, followed by data for systolic afferent arteriolar pressure as measured by imposing various pressures through the ureter. Two glomeruli were being watched; if flow in neither stopped in 10 to 15 seconds an upward arrow was recorded, if one stopped a cross, if both stopped a downward arrow. Male frog *Tu*.

The frog showed the same latency for oliguria as for decrease of heart rate upon administration of carbon dioxide, but when the carbon dioxide was removed the pressure *began* to recover more quickly than urine formation recovered.

The latent periods found in eight tests with 29 per cent carbon dioxide and 71 per cent oxygen were: renal arteriolar pressure began to decrease in 5 minutes; recovery (increase) began in 5 minutes. In seven tests with 11 to 21 per cent oxygen, the decrease of arteriolar pressure began in 4 minutes; recovery began in 2 minutes. These pressure changes were simultaneous with the other changes observed in the circulation (table 3), and therefore at the onset of oliguria were simultaneous with the first decrease of urine production (table 2).

No absolute significance can be attached to these local pressures. It may be mentioned that the mean value found in 29 control determinations upon six frogs that were in an atmosphere of 100 per cent oxygen was 28 mm. mercury. This may conceivably be compared with the average value of 23.5 mm. mercury obtained in afferent arterioles by Hayman (1927) using the method of introducing pressure into a single capsule through a microcannula, until the systolic inflow of blood ceased. The difference is not necessarily due to resistance to pressure transmission through the renal tissues, for the conditions of the animals were not identical (e.g., with respect to oxygen tension and to blood plethora) in Hayman's investigation and in the present work.

The amounts of change in afferent arteriolar pressure were usually greater than those in rate of heart beat. Thus in 11 per cent oxygen the pressure was 87 per cent of the control (3 tests); in 21 per cent oxygen was 78 per cent (4 tests); in 29 per cent carbon dioxide was 77 per cent (9 tests). They were nearly the same as those in mean aortic pressure (table 3). The tests of arteriolar pressure were limited to these agents of group C. It should not be concluded that the arteriolar pressure decreased in proportion to the general arterial pressure in response to all agents. In the instances of visible arteriolar constriction (agents 1 to 6a) such relationship is known not to hold.

Other features of the experiments in which pressure was imposed through the ureter may be mentioned here. In additional instances the cannula and tubing were not filled with saline to the leveling reservoir, but the meniscus was within the cannula. This allowed measurements of the rate of entrance of fluid into the kidney. The fluid entering did not all issue again when the pressure was released, and both the rate of entrance and the amount retained increased with the pressure imposed. But a larger proportion of the fluid was retained (up to 70 per cent of that entering) when high pressures were applied (30 to 35 mm. mercury). The rates of entrance and amounts of retention diminished with the duration of the pressure application, both in the living and in the dead animal. These and other facts illustrate first that the kidney is distensible and elastic, part of the distensibility being due to displacement of blood from the renal blood vessels; and second, that fluid escapes from the urinary passages into the renal tissues, and from the renal tissues into the blood. The amounts of fluid escaping into the kidney amounted to as much as 2 cc. in the course of 4 hours of repeated pressure application, while the kidney finally weighed only 100 mgm. The urine production meanwhile steadily increased with the blood plethora whenever periods for measuring its rate intervened.

Measurements of both aortic pressure and *glomerular* blood pressure were carried out by Hayman (1927) under normal conditions and in the presence of epinephrine and of intravenously injected saline. In some instances

diminution of these pressures after epinephrine injection was reported, but these were not in the majority. Obviously proper correlations may be found only by measuring the glomerular blood pressures under circumstances in which the effects on rates of urine output are known.

Summary of results. In response to each of the thirteen agents (groups A to D) that produced oliguria, there was some circulatory change. Three agents (group E) that did not interfere with urine formation left the circulation unaffected. No means was found of depressing the circulation without inhibiting urine excretion.

No response but oliguria occurred in any test of the thirteen agents reported to produce it, with two sorts of exceptions: epinephrine sometimes (9 out of 23 tests) brought on polyuria without the oliguric phase, as detailed in another paper (1936); and, in two tests not reported, polyuria was induced by an atmosphere of 21 per cent oxygen replacing 100 per cent oxygen.

COMMENT. To discover and study *all* the agents that can diminish the rate of urine formation in the frog is obviously an unmanageable task. But 16 agents represent a liberal sample; and since 13 out of 13 oliguric agents act primarily on the circulation, the expectation that the next oliguric agent tested would have no circulatory action should be very small, though it is incapable of statistical expression.

The question naturally arises whether several of the agents used to produce oliguria may not mediate their effects through some common factor. Thus, epinephrine might be liberated through the agencies of pituitary extracts, absence of oxygen, and other influences. In such a case several of the agents used would merely illustrate the effects of one final agent. There is no direct evidence that this is or is not the case.

Richards (1929) observed in frogs injected with mercuric chloride 24 hours previously that glomerular fluid was forming in certain capsules, while no urine was issuing from the ureter. His explanation was therefore that fluid was passing from the blood into the capsules at normal rates, but later completely escaped because of loss of selectivity of the tubular epithelium. The circulations have not been studied in frogs rendered anuric for 24 hours. The rates of urine formation by intact frogs have now been measured, however, and the occurrence of anuria for 24 hours or longer when large doses of mercuric chloride were injected subcutaneously was confirmed. Frogs whose anuria persisted for such a long period rarely recovered.

It is impossible to affirm that the agents used to induce oliguria have no action in the kidneys other than that upon the circulation. The general fact which emerges is that the effects upon the circulation are of such a nature, of such a magnitude, and occur at such a time as to account for the oliguria found.

The observations favor the supposition made by Ludwig (1844) that the pressure of the blood in the glomerular capillaries is effective in expelling water from the blood to form urine. Whatever may occur in the tubular structures of the kidney does not appear to interfere with the parallelism between glomerular condition and urine production, in spite of the fact that the absolute quantities of urine may still be modified by the tubular functions.

The question might arise why the conditions for polyuria were not studied along with those for oliguria. It is obvious, however, that the methods of observing the glomerular circulation were not adequate for detecting increases in it. Marked diminution of blood flow in glomeruli can be ascertained consistently, but augmentations would require quantitative measurement. Decreases of arterial pressure are almost certain to involve decreases of local blood pressure, but increases may easily be cut off by the interposed arterioles.

SUMMARY

1. Thirteen methods of producing decreases of urine formation in the frog were studied; all decreased the circulation of blood either locally or generally. Three additional agents that produced no oliguria produced also no circulatory changes.

2. Either one or both circulatory factors that were observed (the local glomerular blood supply or the general arterial pressure) correlated in degree with the rate of urine formation. In many instances the general circulatory condition could be judged by comparison of rates of heart beat; in others measurements of arterial blood pressure were required.

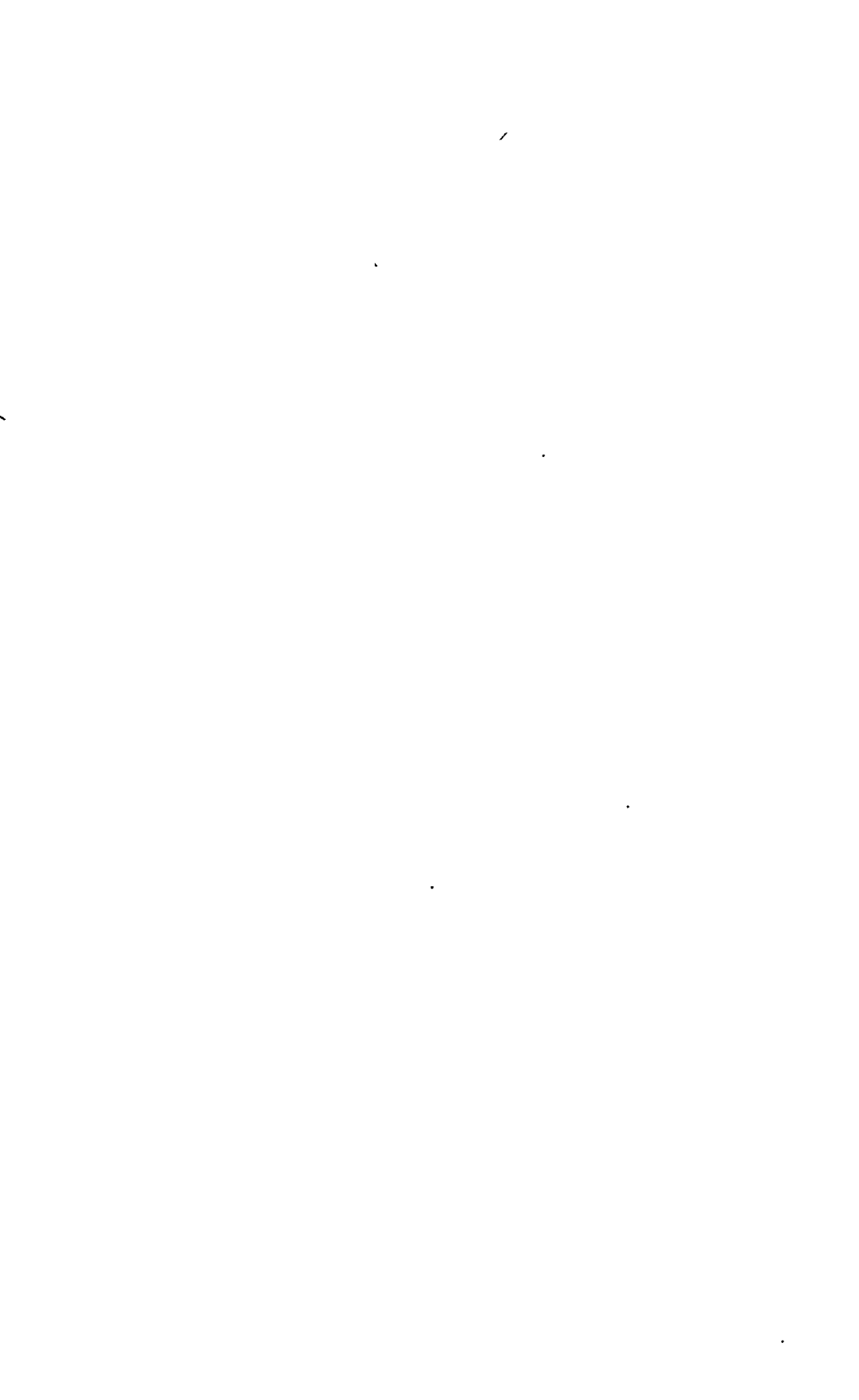
3. The latent periods of circulatory change were similar to those for the onset of oliguria; but in the recovery from oliguria the excretory rate might be restored some time after the circulation improved.

4. Since no oliguric agent was found that did not act on the circulatory system, it is suggested that urine formation was diminished in all the cases studied by decrease of mean blood pressure within glomerular blood vessels.

REFERENCES

- ADOLPH, E. F. This Journal 108:177, 1934.
This Journal 111:64, 1935a.
This Journal 111:75, 1935b.
This Journal 115:200, 1936.
BIETER, R. N. AND F. H. SCOTT. This Journal 91:265, 1929.
CULLIS, W. C. AND E. M. SCARBOROUGH. J. Physiol. 75:33, 1932.
HAYMAN, J. M., JR. This Journal 79:389, 1927.
HILL, L. AND J. McQUEEN. Brit. J. Exper. Path. 2:205, 1921.
HOFMEISTER, F. Pflüger's Arch. 44:360, 1889.

- KUNO, Y. Pflüger's Arch. 158:1, 1914.
- LUDWIG, C. In R. WAGNER's Handwörterbuch d. Physiol. 2: 628, 1844. Braunschweig, Vieweg and Sohn.
- RICHARDS, A. N. Trans. Assoc. Am. Physicians 44: 64, 1929.
- RICHARDS, A. N. AND C. F. SCHMIDT. This Journal 71: 178, 1924.
- RICHARDS, A. N. AND A. M. WALKER. Am. J. Med. Sc. 190: 727, 1935.
- SCHULZ, F. N. Pflüger's Arch. 115: 386, 1906.
- TAMURA, K., K. MIYAMURA, T. NISHINA, H. NAGASAWA AND M. HOSoya. Jap. J. M. Sc. Tr. Pharmacol. 1: 229, 1927.



THE AMERICAN JOURNAL OF PHYSIOLOGY

VOL. 117

NOVEMBER 1, 1936

No. 3

OVARIAN HORMONE THRESHOLD FOR EXPERIMENTAL MENSTRUATION IN MONKEYS

EDGAR ALLEN, A. W. DIDDLE, T. H. BURFORD AND W. U. GARDNER

From the Department of Anatomy, Yale University¹

Received for publication April 24, 1936

The appearance of the first menstrual period, because it is such a clear-cut and obvious event, serves as the best criterion of sexual maturity in female primates. Menstruation may be induced experimentally, even in infant monkeys, by short treatment with ovarian follicular hormone. Determination of the amount of this hormone necessary to produce menstruation as immature animals approach sexual maturity should furnish a hormone threshold for adolescence of greater value than assays of urine for excreted hormone.

Other adolescent changes, such as development of the mammary glands, might be used as indicators, but these changes are gradual. The first ovulation, if it were possible to easily determine the time of this event, would probably be the final criterion of sexual maturity. In primates, however, ovulation occurs with so little outward change that until recently its time in the menstrual cycle has been much disputed.

In monkeys the first clear sign of approaching sexual maturity is the reddening of the "sexual skin," that of the region immediately surrounding the genital organs (1). Gradually the color deepens, the area affected spreads and the skin begins to swell. This reddening and swelling may fluctuate in degree for several months before the onset of the first menstrual period. Then follow the first menstrual cycles, the onset of menstruation usually being preceded by decrease in the intensity of the reddening of the "sexual skin" (2). These local cutaneous changes have been induced in ovariectomized monkeys by injections of ovarian follicular hormone (2), and therefore they are clearly indicative of increase in level of this hormone and offer an accurate criterion of effect of injected extracts.

¹ This work was supported by a grant from the Committee for Research in Problems of Sex of the National Research Council.

The following experiments attempt to find the amount of follicular hormone and the optimum time of treatment for the experimental production of menstruation in monkeys of different ages—immature, adolescent, and adult.

EXPERIMENTAL PROCEDURE. Seventeen female monkeys (*Macacus rhesus*) have been ovariectomized for this study. Of this group seven were mature, five adolescent, and five immature. Classification was made on a basis of 1, body weight; 2, presence or degree of reddening and swelling of the "sexual skin"; 3, size of the uterus, and 4, presence or absence of large follicles and corpora lutea at the time of ovariectomy.

After a post-operative recovery period, injections of water-soluble or oil-soluble hormone (theelin or amniotin)² were begun. Injections were given twice daily (8:30 a.m. and 4:00 p.m.) in an effort to maintain as nearly as possible uniform levels of hormone. The total dosages ranged between 78 and 2250 rat units, and the time of hormone treatment between 4 and 22 days.

The intensity of reddening of the "sexual skin" was followed as an indicator of the effectiveness of the hormone action. It appeared or became intensified after several days of injections. Following the cessation of injections the animals were observed closely for the onset of bleeding and records kept of the latent period; i.e., the time elapsing between the cessation of injections and the appearance of bleeding, and also of the duration of the flow. When it was certain that menstruation had ceased (or was not going to occur) injections were resumed. In all instances menstrual bleeding was observed externally. Bleeding so slight as to require microscopic identification of red blood cells in vaginal lavages was not recorded.

OBSERVATIONS. A total of 44 experimental menses was produced in seventeen ovariectomized monkeys of different ages; as many as five, and in one case eight, successive experimental menstrual periods in certain individual cases. In 15 instances the treatment failed to produce menstruation, either because of subthreshold doses of hormone or too short periods of injections. This made a total of 62 experiments.

I. Mature ovariectomized monkeys. These animals weighed from 4100 to 5700 grams at the time of ovariectomy. All had well developed "sexual skin" phenomena, had menstruated regularly, and had ovaries containing corpora lutea or large follicles.

In table 1 are listed experiments with oil soluble extracts of estrogenic material in ovariectomized adults. Animal 1 LE was given four series of injections ranging from 9 to 20 days in length. The first experiment began 31 days after ovariectomy and a total dose of 138 rat units of hormone

² Acknowledgment is made to Dr. Oliver Kamm of the Parke, Davis Company, and to Dr. John F. Anderson of E. R. Squibb and Sons for generously supplying these preparations.

was injected over a period of 20 days. After a latent period of 3 days she menstruated for 3 days. Four days after the first experimental menses had terminated, a second series of injections was given in which the total dose was reduced to 78 rat units during 20 days. Although producing some reddening of the "sexual skin," this treatment was not followed by menstrual bleeding during the following 50 days. This was probably a subthreshold dose over this interval of time. When in the third series of injections a dose only slightly larger (81 rat units) was given in less than half the time (9 days), it was followed by a latent interval of 6 days and then 2 days' menstruation. A fourth series in this animal, 230 rat units in 14 days, failed to produce menstruation.

TABLE 1

*Experimental menses in ovariectomized mature monkeys following injections of ovarian follicular hormone in oil solution**

MONKEY NUMBER	INJECTION SERIES	DAYS INJECTED	TOTAL DOSE (RAT UNITS)	LATENT PERIOD	DURATION OF MENSES
1 LE	1	20	138	3	3
	2	20	78		None
	3	9	81	6	2
	4	14	230		None
2 RE	1	20	78		None
	2	9	122	7	2
	3	14	117	4	3
	4	10.5	138	3	1
3 N	1	14	95	4	1
	2	14	190	5	6

* In these experiments doses were gradually increased to a maximum 4 or 5 days before cessation of injections.

The details of similar experiments in animals 2 RE and 3N may be followed by reference to table 1.

It will be noted that in two instances 78 rat units of oil-soluble extract in 20 days proved to be a subthreshold dose, while 81, 95, 117, 122 rat units (and larger doses), given over an interval of 9 to 14 days, proved adequate. In the above experiments extracts were dissolved in oil, which would favor slow absorption and also gradual fall in hormone level after cessation of injections.

In table 2 are listed experiments with a water soluble preparation of theelin, also in adult ovariectomized monkeys. For purposes of standardization of treatment, the daily dosage was maintained at the same level throughout the experiment. Injections were made twice daily.

Monkey 5 M is discussed briefly as a "sample" of this group. She had 5 successive experimental menstrual cycles. For the first three, the daily dose of 50 rat units was maintained, but the period of injections varied from 15 to 8 and 6 days, with correspondingly decreasing total doses. The post-injection period of 16 days before the onset of menses was the longest latent period, and the resulting menstrual period the shortest, observed in any of our experimental animals.

In monkey 5 M the first measurement of the uterus (24 x 18 mm.) was taken at the time of ovariectomy, which was done shortly after ovulation

TABLE 2

*Experimental menses in ovariectomized mature monkeys following injections of ovarian follicular hormone**

MONKEY	EXPERIMENT	DAYS BETWEEN LAST MENSES AND NEXT TREATMENT	HORMONE INJECTED (RAT UNITS)		DAYS INJECTED	LATENT PERIOD	DURATION OF MENSES
			Daily	Total			
4 M	1		100	2200	22	5	6
	2		100	400	4		None
	3	1	100	600	6	Died	
5 M	1	15	50	750	15	6	6
	2	1	50	400	8	6	5
	3	1	50	300	6	16	1
	4	1	15	150	10	11	2
	5	5	40	400	10	9	3
6 M	1	15	150	2250	15	6	7
	2	1	150	1350	9	8	6
	3	1	30	180	6	10	3
	4	2	15	150	10		None
	5	30	40	400	10	6	5
7 M	1	1	30	300	10	6	5

* In these experiments injections were maintained at a uniform level throughout.

had occurred (table 3). One hundred and thirty-seven days after ovariectomy and 2 days following cessation of the fifth series of injections the uterus was slightly smaller (22 x 18 mm.). A third measurement (25 x 20 mm.) made on the last day of treatment, 21 days after the second laparotomy, showed the uterus larger than before ovariectomy. The hormone treatment given was adequate, therefore, to prevent involution of the uterus over a relatively long period (158 days).

A large dose and a long treatment (5500 rat units in 15 days) was given ovariectomized adult 6 M for her first series of injections and this was followed by a second series much above the minimal level. Unusually long

menses, 7 and 6 days respectively, with profuse flow, followed latent periods of 6 and 8 days. Reduction of dosage and time of treatment in her third series of injections was followed by increase of latent period and decrease of duration of flow. Further reduced dosage in the fourth series failed to produce menstruation.

The uterus of animal 6 M showed very little change in size over a period of 162 days (table 3). In other words, a total dose of 4,330 rat units of theelin alone, given in 5 treatments totaling only 50 days of the 162 day period (a total of 112 days without hormone), was sufficient to maintain this adult uterus at its "ovulation time" level.

In summarizing results from this series of injections of water-soluble follicular hormone in ovariectomized adults, it is clear that 180 rat units

TABLE 3
Measurements of uteri

GROUP	MONKEY NUMBER	SIZE AT OVARECTOMY	INTERVAL BETWEEN MEASUREMENTS	SIZE AT 2ND MEASUREMENT*	INTERVAL IN DAYS BEFORE NEXT MEASUREMENT	SIZE AT TERMINATION OF INJECTIONS
		mm.				
Immature	13 I	12 x 8	110	12 x 8		
	16 I	14 x 5	87	15 x 6		
	17 I	10 x 5	87	12 x 9		
Adolescent	8 A	20 x 17	146	20 x 16	22	20 x 17
Mature	4 M	26 x 20	90	20 x 9		
	5 M	24 x 18	137	22 x 18	21	25 x 20
	6 M	22.5 x 17	140	22 x 15	22	23 x 17

* At laparotomy in animals 5 M, 6 M, and 8 A; after the last injection in animals 4 M, 13 I, 16 I, and 17 I.

of hormone (in one case 150) equally distributed in 10 days, was above the hormone threshold for menstruation. Smaller doses of oil soluble hormone (81 r.u.) were effective. Also the hormone level sufficient to produce menstruation was adequate to maintain the uterus at its size at the time of ovariectomy and to prevent "castrate" atrophy of the mammary glands in adults, for the mammae of the injected monkeys were equivalent to those of fully developed normal, non-pregnant animals.

II. *Adolescent ovariectomized animals.* The 5 animals of this group showed reddening and swelling of the "sexual skin" but had not menstruated, and at the time of ovariectomy the size of the uterus and absence of corpora lutea or large follicles in the ovaries clearly indicated that they should be classed as approaching sexual maturity (table 4). The body weight ranged from 3300 to 4600 grams.

In animal 8 A the first 3 experimental periods were begun with a daily dose of 100 rat units and the periods of injections were 22, 3, and 6 days respectively. In the first series, the injection of 2200 rat units of hormone was followed by a latent period of 6 days and profuse menstruation lasting for 6 days. The day after cessation of this menstruation the same daily dosage was given for three days. Observations for 14 days thereafter failed to disclose any bleeding. It is probable that 3 days is too

TABLE 4

*Experimental menses in ovariectomized adolescent monkeys following injections of ovarian follicular hormone**

MONKEY	EXPERIMENT	DAYS BETWEEN LAST MENSES AND NEXT TREATMENT	HORMONE INJECTED (RAT UNITS)		DAYS INJECTED	LATENT PERIOD	DURATION OF MENSES
			Daily	Total			
8 A	1		100	2200	22	6	6
	2	1	100	300	3	(14)	None
	3	14	100	600	6	9	4
	4	6	40	400	10	10	2
	5	6	60	600	10	8	2
9 A	1		30	450	15	6	2
10 A	1		40	400	10	10	3
	2	2	25	250	10		None
	3	58*	30	300	10	(14)	None
	4	83*	40	400	10	8	1
	5	15	35	350	10	10	1
11 A	1		30	300	10	9	4
	2	1	30	300	10	7	3
	3	50	25	250	10	7	3
	4	7	20	200	10	6	2
	5	17	20	200	10	7	1
12 A	1		30	300	10	(15)	None
	2	15*	30	300	10	6	2

* Or after last injection if treatment failed to produce menstruation (see column 8).

short a time to induce sufficient growth of the endometrium. The same daily dose in the third series, 100 rat units for 6 days, produced a menstrual period of 4 days but the latent period was increased to 9 days. The uterus measured approximately the same at the time of the last injection, 168 days after ovariectomy, as at the time of the ovariectomy.

Monkeys 10 A and 11 A are of special interest, as 5 successive series of injections were made retaining a 10 day period of injections in each case.

After 400 rat units had produced a menstrual period of 3 days in monkey 10 A, a second series of 250 units failed to produce menstruation. In the third experiment begun 58 days later, 300 rat units also failed to produce menstruation. A fourth experiment with a total dose of 400 rat units resulted in menstruation for one day after an 8 day latent period. When the experimental menses lasted for a day only, the flow was usually slight. In all cases, however, it was readily apparent without need for identification of red blood cells by the lavage method. In adolescent animal 11 A, dosages of 200 to 300 rat units in 10 days were clearly above menstrual threshold. It will be noted that duration of menstrual flow decreased with decreasing doses of hormone.

From these experiments it was concluded that a 3 day period of injection is too short a time (monkey 8 A), that 6 days may be adequate, but that probably 10 days of injections are nearer optimal for growth of the endometrium which apparently must precede menstruation. As the dosage is decreased there is a tendency for the resulting menstrual periods to be shortened and the amount of flow to be decreased. As in the previous experiments in adult monkeys, measurements of uteri from several of these adolescent monkeys at the end of injections show this dosage adequate to maintain, or in some cases enlarge, the uterus, even though considerable periods without hormone were interspersed between treatments.

III. *Immature ovariectomized animals.* Five definitely immature monkeys were ovariectomized for a fourth series (table 5). The body weight of 1500 to 1950 grams clearly indicated that they were much too young to be approaching adolescence. There was no reddening or swelling of the "sexual skin" and at ovariectomy both uterus and ovaries were distinctly infantile.

Animal 13 I is noteworthy as she was given 8 successive series of injections, in most instances 10 days in length. Total doses ranged from 300 to 1000 rat units. In the first series 780 rat units in 13 days produced a menstrual period of 4 days after a latent interval of 7 days. When the dose was dropped to 600 rat units and the injection period reduced to 10 days, the latent interval was increased to 10 days but menses lasted for 5 days. In the third series treatment for 6 days was effective. Other details are easily followed by study of table 5. In the last experiment 300 rat units in 10 days failed to produce menstruation. It is probable that this is a subthreshold dose for such an immature monkey.

On the day following cessation of treatment in the eighth series the uterus was found to measure the same as at ovariectomy (12 x 8 mm.), which was done 110 days previously.

TABLE 5

Experimental menses in ovariectomized immature monkeys following injections of ovarian follicular hormone

MONKEY NUMBER	TREAT- MENT	DAYS BE- TWEEN LAST MENSES AND THEELIN TREATMENT*	HORMONE INJECTED (RAT UNITS)		NUMBER OF DAYS INJECTED	LATENT PERIOD	DURATION OF MENSES
			Daily dose	Total dose			
13 I	1		60	780	13	7	4
	2	1	60	600	10	10	5
	3	1	60	360	6	10	3
	4	1	35	350	10	9	2
	5	6	60	600	10	6	3
	6	153	100	1000	10	9	3
	7	2	35	350	10	6	2
	8		30	300	10	12	0
14 I	1		30	300	10	14	0
	2	14	30	300	10	5	2
	3	1	25	250	10	14	0
15 I	1		30	300	10	14	0
	2	14	30	300	10	5	2
	3	1	20	200	10	14	0
16 I	1		30	330	11		0
	2	17	30	300	10	9	4
	3	13	60	600	10	7	4
17 I	1		15	165	11		0
	2	17	15	150	10		0
	3	25	60	600	10	7	4

Monkeys 14 I and 15 I were tried for 2 experiments in each with the same hormone level and injection period. The first treatment failed to produce menstruation in each case, while the second series was effective. This is similar to the results obtained in monkey 12 A (table 4). It is probable, therefore, that in the first experiment after ovariectomy 300 rat units in 10 days is definitely below the menstrual threshold for animals of this age. A third series of 200 rat units in monkey 15 I and 250 units in 14 I failed to induce menstruation. It seems definite that these are sub-threshold doses for immature animals as far as the production of menstruation is concerned.

In animal 16 I a first experiment with a dose of 330 rat units failed to produce menstruation. Seventeen days after the last injection, the second treatment with 300 rat units was effective. This result is similar to the second series in animals 14 I and 15 I. There may have been some cumulative effect from the first treatment.

In animal 17 I, 165 and 150 rat units were clearly subthreshold doses.

The uteri of monkeys 16 I and 17 I on the day following discontinuation of the third series of hormone injections and 87 days after ovariectomy, were larger than at the time of ovariectomy (table 3).

In summary for this group it can be said that the menstrual threshold of ovarian follicular hormone in immature monkeys (300 r.u. positive 3 times, negative 3 times) is considerably higher than that in adult and adolescent monkeys. It is remarkable, however, that the uterus in such an immature condition reacts by menstruation after such brief treatment with follicular hormone. Some growth of both uterus and mammary glands was apparent at the end of injection experiments, in spite of the intervals without hormone between periods of treatment.

DISCUSSION. Examination of the ovaries of monkeys after the first few menstrual cycles indicates in most cases that ovulation has not occurred, thus establishing menstruation without ovulation and corpus luteum formation as a common occurrence in adolescent monkeys (3) (4) (5). The anovulatory menstrual cycle is also the usual thing during the summer months in adult monkeys. Menses in these anovulatory cycles cannot be distinguished externally either in duration or amount of flow from those which have been preceded by ovulation. The vascular reactions as observed in menstruating endometrium in ocular implants are the same in both ovulatory and anovulatory cycles (6). The only sure method of distinguishing is by histological recognition of small bits of endometrium, but it is necessary to curette to make this diagnosis.

Menstruation has been produced experimentally in normal adult monkeys by destroying large follicles (2) (7), by removing one or both ovaries (2), by removing recent corpora lutea (8), or by sectioning nervous pathways to the ovaries either in the spinal cord or peripherally (9) (10).

Menstruation has been produced from interval endometria in ovariectomized monkeys by injections of ovarian follicular hormone (2) (4) (11) (12) (13). These studies have shown that the principal action of the injected hormone is to induce growth in the endometrium and that a certain amount of this is necessary before menstruation can occur. After injections are stopped, a latent period of several days intervenes and then menstruation sets in. These experimental menses are indistinguishable externally from normal menses. Werner and Collier (1933) and others have reported similar experimental menstrual periods with the use of theelin alone in ovariectomized women.

When the follicular hormone is followed by short treatment with the corpus luteum hormone, the endometrium undergoes transformation equivalent to that of the premenstrual stage of ovulatory cycles and then menstruates when treatment is stopped. Recently Engle and Smith (14); Hisaw (15); Engle, Smith, and Selesnyak (16); and Corner (17) have described postponement of the expected menstruation either in normal

animals or in theelin treated animals by injections of corpus luteum hormone. Although the corpus luteum may have this endocrine function as regards menstruation in the ovulatory cycle, the evidence cited above for anovulatory menstrual cycles and for menses induced experimentally by theelin injections in ovariectomized primates remains fundamental.

Menstruation has been the subject of several recent reviews to which reference is made for further discussion (18) (19) (20).

While it may be desirable for purposes of standardization to inject the same daily dose over a period of days, it would probably approach much nearer conditions of normal secretion of follicular hormone by the ovaries to begin with moderate doses and increase the amount to a maximum as in the first series of injections (table 1). This method should simulate normal conditions of ovarian secretion as follicles approach maturity.

It seems clear that 3 or 4 days at the hormone levels tested is too short an interval to produce the necessary growth changes in the uterus to put it into condition where menstruation will begin when the hormone is withdrawn. A period of 6 days may be adequate, but a longer period of injections reduces the latent period before onset of menstruation and more nearly approaches the time relations of the normal menstrual cycle.

Sometimes a dosage and time interval which is not effective in the first experiment in immature and adolescent animals may prove effective in a second experiment on the same animals. It is possible that there is some cumulative effect or that an effect on other related endocrine glands, perhaps the anterior-pituitary, may be carried over to the next experimental cycle.

As there were intervals of complete absence of hormone between successive series of injections, these experiments did not reproduce the condition in a normal animal during several successive anovulatory cycles. It is possible in the normal animal that there is always some ovarian hormone present, but that the amount fluctuates. For this reason maintenance of continued growth of mammary glands and uterus is hardly to be expected. It is worth emphasis in this connection, however, that the size of the uterus is maintained by the hormone treatment described;—the measurements of the uteri taken at the termination of injections were in all cases equal to, or greater than, those made at the time of ovariectomy. Also this hormone treatment maintained the adult condition of the mammary glands in the older animals and induced considerable growth of mammary duct systems in the younger animals.

It is probably not desirable to attempt to calculate on the basis of body weights clinical dosages of ovarian hormone from these results in monkeys. It seems reasonable, however, that much smaller doses than re-

ported recently in the clinical literature would be effective as therapeutic measures clinically. From studies of atypical growths resulting from massive doses of estrogenic substances in both mice and monkeys it would seem preferable to hold therapeutic doses to as near the effective threshold as possible (21).

SUMMARY

Menstruation is one of the best criteria of adolescence in primates. This study reports the amount of ovarian follicular hormone (theelin, amniotin), and time of treatment, necessary to induce experimental menses in ovariectomized monkeys of various age groups.

In ovariectomized adults small doses of hormone "condition" the uterus (produce an interval endometrium) so that cessation of injections is followed by menstruation. Larger amounts of hormone are necessary to produce menstruation in adolescent and immature monkeys than in mature ones. Large doses of theelin, when concentrated in periods of time less than 6 days, are less effective. It is probable that the minimal time for the establishment of theelin effects approximates 6 days and the optimum 10 or more.

When theelin treatment is concentrated in shorter periods than 10 days, larger doses are required and longer latent periods transpire before menstruation sets in. The duration and amount of flow are likewise diminished by decreasing the dosage and shortening the period of injections.

A dosage which proves adequate for the menstrual threshold maintains the size of the uterus and induces growth in the mammary glands, even though periods without hormone are interspersed with periods of injections.

REFERENCES

- (1) ALLEN, E. *J. Morphol.* 46: 479, 1928.
- (2) ALLEN, E. *Contrib. to Embryol.* (no. 98), Carnegie Inst. of Washington 19: 1, 1927.
- (3) CORNER, G. W. *Contrib. to Embryol.* (no. 75), Carnegie Inst. of Washington 15: 73, 1923.
- (4) ALLEN, E. *Am. J. Anat.* 42: 467, 1928.
- (5) HARTMAN, C. G. *Contrib. to Embryol.* (no. 134), Carnegie Inst. of Washington, Pub. 433, 1932.
- (6) MARKEE, J. E. *Anat. Rec. (Suppl.)* 64: 32, 1936.
- (7) VAN WAGENEN, G. AND S. B. D. ABERLE. *This Journal* 99: 271, 1931.
- (8) PRATT, J. P. *Endocrinol.* 11: 195, 1927.
- (9) VAN WAGENEN, G. *Anat. Rec.* 52: 40, 1932.
- (10) ZUCKERMAN, S. *Anat. Rec.* 58: (Suppl.) 43, 1934.
- (11) MORRELL, J. A., H. H. POWERS, J. R. VARLEY AND J. DEFRATES. *Endocrinol.* 14: 174, 1930.
- (12) SAIKI, S. *This Journal* 100: 8, 1932.
- (13) SMITH, P. E. AND E. T. ENGLE. *Proc. Soc. Exper. Biol. and Med.* 29: 1225, 1932.

- (14) ENGLE, E. T. AND P. E. SMITH. Anat. Rec. 61: 471, 1935.
- (15) HISAW, F. L. Am. J. Obstet. and Gynec. 29: 638, 1935.
- (16) ENGLE, E. T., P. E. SMITH AND M. C. SHELESNYAK. Am. J. Obstet. and Gynec. 29: 787, 1935.
- (17) CORNER, G. W. This Journal 113: 238, 1935.
- (18) ALLEN, E. Chap. XII, Glandular physiology and therapy. A. M. A. Press, 1935.
- (19) ALLEN, E. Chap. IX, Sex and internal secretions. Williams & Wilkins Co., Baltimore, 1932.
- (20) CORNER, G. W. Medicine 12: 61, 1933.
- (21) Editorial. J. A. M. A. 106: 1093, 1936.

THE SITE OF ACTION OF BOTULINUS TOXIN

GEORGE H. BISHOP¹ AND JACQUES J. BRONFENBRENNER

From the Laboratory of Neurophysiology and Department of Bacteriology, Washington University School of Medicine, St. Louis

Received for publication May 28, 1936

The toxin of botulinus on administration to animals produces after a definite incubation period a paralysis of skeletal musculature, usually resulting in death by paralysis of the respiration. The incubation period is prolonged, or the onset of symptoms delayed, by the administration of anesthetics (Bronfenbrenner and Weiss, 1921, 1922, 1924). This might suggest an involvement by toxin of the nervous system as one of the obvious sites of anesthetic action, in spite of good evidence in the literature of a curare-like action on the periphery. We have therefore reexamined the action of this toxin with special reference to the central nervous system, as a preliminary to the study of the effect of anesthetic.

After a comprehensive study of the toxin's action on frogs, dogs, etc., Edmunds and Long (1923) and Edmunds and Keiper (1924) decided that the action was curare-like, in that it left the muscle still directly excitable, but inexcitable indirectly through its end-plate. Our own results confirm this. The present work involves the further test of botulinus toxin from two angles. First, a relatively pure and high-potency toxin permitted massive dosage with a minimum of the action assignable to impurities or contaminants. Second, the electrometric recording of nerve responses has reached a stage where a direct effect on the nervous system could be detected if it were produced. The definitive action of the toxin still appears to be curare-like, at the myoneural junction. There also is an effect of large doses on the heart and on the central nervous system, which presumably indicates that like most agents with a specific biological action, the specificity should be put in terms of threshold. As a potentially general biologically injurious agent, the point of greatest susceptibility, or of injury by least dosage, is the myoneural junction.

Preparation and dosage of toxin. The toxin used in the experiments was prepared by growing a culture of *B. botulinus* type A on a 1 per cent glucose broth poured over an equal volume of finely ground beef heart tissues. At the end of 4 days of growth under anaerobic conditions the

¹ Assisted by a grant-in-aid for Research in Neurophysiology from the Rockefeller Foundation.

liquid portion of the culture was poured off, filtered through the Berkefeld "N" candle, and preserved in sealed tubes under CO_2 . The toxicity of this filtrate was determined by intraperitoneal injection of mice weighing 18 to 21 grams, and the amount which killed these mice in approximately 48 hours was assumed to be a minimal lethal dose.

Over a period of several months while the work was in progress, this toxin was retitrated periodically and the minimal lethal dose was found to vary from 0.000003 to 0.00001 cc. As a rule, 250 mouse lethal doses of toxin were needed to kill a 2000 gram rabbit in 24 hours by intravenous injection. The killing effect on rabbits, however, was much less regular than in mice, for reasons that will appear below.

Apparatus. The apparatus employed for maintaining respiration is diagrammed in figure 1. *A* is a tin can of 6-inch diameter, with a movable disc *B* operated by a rod *D* through the end to adjust for various sized rabbits. A ring *C* fits the other end, sealing to *A* by a gasket and held by three wing nuts, their bolts being hinged for quick tightening in an

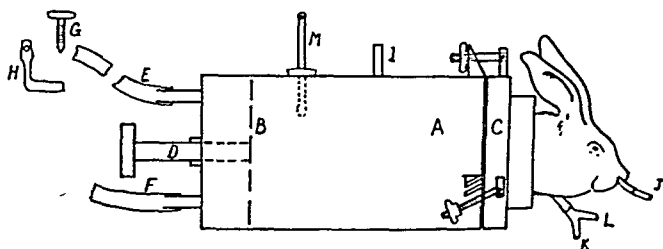


Fig. 1. Diagram of apparatus for artificial ventilation of paralyzed rabbits. Explanation in text.

emergency. This ring has tied over it the wrist of a rubber glove, the fingers cut off to leave a tube, through which the rabbit's head and one fore leg are pushed before applying *C* to *A*. *E* and *F* are rubber tubes connecting to two pairs of valves, one of each pair, *G*, being a needle valve to regulate rate of air flow, the other, *H*, a cut-off valve to regulate duration of opening. The latter valves *H* are operated by a motor driven eccentric, with such adjustments that the extreme position of opening, the length of stroke (and thence duration of open period) and the frequency of opening are separately regulated, and the two valves can be set to open at any phase relationships. A line from one valve runs to the vacuum supply, the other can be left open or carried to the pressure line. A side tube *I* can be used to record the pressure changes on a tambour, or when the respiratory machine is stopped, to record the animal's natural respiration. The insertion of the rabbit and the starting of the apparatus took about one-half minute, which in some cases proved too

long. The rabbit's fur was greased with vaseline, or adhesive tape applied, to seal the rubber sleeve. A thermometer was inserted at *M*.

Animals could be maintained in such an apparatus, after the failure of natural respiration, until accident intervened, with occasional administration of water or glucose solution. Usually the nostrils plugged with mucus, and glass tubes were inserted as at *J*, which required frequent cleaning. It was found more satisfactory, after the first few hours, or before an emergency arose, to insert a glass tracheal cannula, which could be done under ether without removing the animal from the respirator. A large opening at *K* permitted removal of mucus from the trachea with a pipe cleaner, and a side tube *L* served to record respiration by a tubulature to a bellows recorder, the aperture *K* being narrowed just sufficiently to give the desired sensitivity. During tests of natural respiration when the latter had nearly ceased (after apnea by artificial ventilation, or after partial paralysis), the complete closing of *K* allowed the pulse rate to be clearly recorded as a test of heart action.

EXPERIMENTAL. After the injection by ear vein of up to 3000 mouse M.L.D.'s of toxin per kilo, no perceptible symptoms are exhibited during the incubation period; after the first symptoms, of uneasiness and startled appearance, the train of events is the more rapid the larger the dose, but otherwise largely independent of dosage. Death in rabbits, without respiratory aid, is almost never due to failure of the primary respiratory musculature, but rather to inability on the part of the animal to open its external nares reflexly for inspiration. The rabbit, at least when intoxicated with botulinus toxin, cannot breathe through its mouth, even when the tongue is drawn forward and the jaws propped open. The external nares are closed passively by cartilaginous flaps, presumably to exclude dust and dirt during burrowing. The action of the toxin, involving difficulty in expelling mucus accumulations from the nasal passages, results in the accumulation of a partially dried viscous glue, which at an early stage of the weakening of the nasal musculature effectually seals the nostrils and the animal very promptly suffocates.

If asphyxia develops slowly, the stage of increased stimulation by oxygen lack is passed over, and depression results immediately. At this stage, a short period of artificial ventilation may revive a completely prostrate animal to the extent that for 15 minutes or less he breathes with sufficient effectiveness to appear almost normal; that is, increased oxygen restores the ability of the respiratory centers to function after the direct impairment of the peripheral mechanism has indirectly depressed the centers. Finally, after all respiratory movements have ceased, the animal can be kept alive by artificial ventilation, until some accidental interference with the artificially manipulated oxygen supply causes death.

At various stages after the failure of natural respiration to maintain adequate supply of oxygen, the responses of the animals have been tested, under ether anesthesia when necessary, and the results compared with the responses of normal animals under the same external conditions.

1. *Failure of respiration.* The respiration, normally rapid and shallow, slows before other signs of respiratory embarrassment are obvious, due to reduction of intake volume rather than to toxic effect on the respiratory center. With progressive slowing, the respiratory efforts become *weaker*, due to paralysis, rather than becoming stronger as they would in normal animals due to a corresponding degree of asphyxia. Finally the animal, now prostrate, ceases to breathe at all, ending with slow gasps accompanied by opening of the mouth and slight movements of the nose without significant movement of air. Terminal asphyxia results suddenly when the tidal air fails to ventilate the alveoli, and asphyxial depression of the center is added to toxic paralysis.

If just before death the animal is resuscitated, a fair degree of recovery of movements occurs. In other words, the central mechanism has been depressed by asphyxia rather than by the toxin. This course of events suggested the experimental procedure of recording at half-hour intervals the respiratory force and rate, and heart beat, of the intact animal, after artificial ventilation just sufficient to produce apnea, allowing the animal to proceed from this condition through successive stages of asphyxia to complete central paralysis, whereupon artificial ventilation was again applied. After even massive doses have resulted in an initial prostration that certainly, if not interfered with, would have caused immediate death, slight respiratory movements can be recorded after ventilation for a period of from six to twelve hours. After smaller but still fatal doses, respiratory movements can be detected in certain animals for at least two days. Often after no movement of air can be recorded even directly from the tracheal cannula at such a sensitivity that the heart beat is recorded via the lung passages with a stroke of the recording lever 3 mm. high, slight movements of the nose such as had previously been associated with inspiratory effort can still be observed. Just before this stage, and after long periods of adequate ventilation, it is found in some animals that the respiratory center (as indicated by the frequency and total number of respiratory efforts between apnea and asphyxia) is still behaving nearly like the normal under comparable conditions, although the peripheral effect of its activity on the musculature is almost zero.

In other cases, however, but only after heavy dosage, the center is probably affected. In these cases, after apnea, the respiratory efforts are abnormally infrequent, may approach the apneustic in type, and cease after a shorter period of asphyxia than previously in the same animal. In two cases we have observed further a complete dissociation

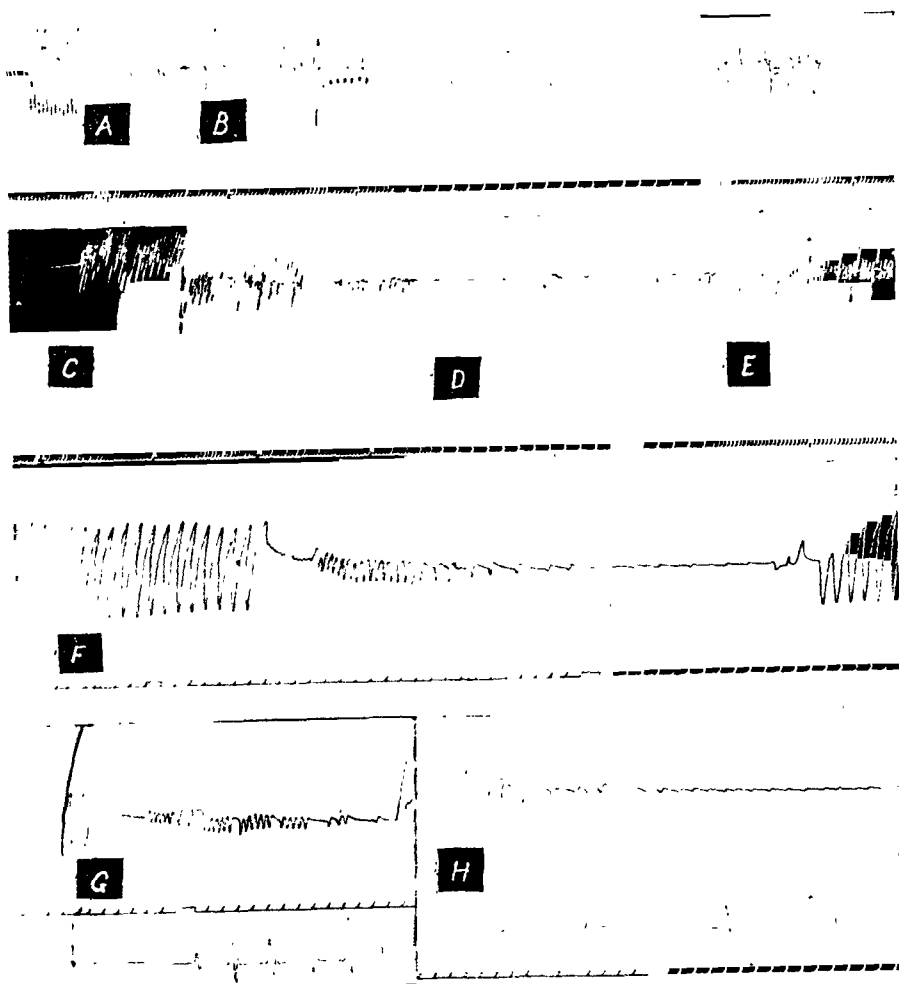


Fig. 2. *A*, artificial ventilation recorded from tracheal cannula, about 1 hour after first prostration of rabbit from respiratory paralysis, 100 M.L.D. intravenous. Two sizes of side-opening of cannula employed. *B*, ventilation stopped, side tube adjusted, record of natural respiratory movements. Large flings of lever are due to jerks of the animal's body. The pulse can be seen on the respiratory record in the fast period of the record. *C*, two hours after *B*, irregular natural respiration after apnea. The two large deflections are body movements. The regular strokes are normal respirations, with gasping or apneustic type movements interspersed, the latter associated with nose movements. *D*, faster kymograph speed, mostly gasping respirations persisting. *E*, artificial respiration restored, after opening side tube; gasping respirations superposed on this, with increased force as ventilation proceeds.

F, three hours after *B*, ventilation stopped, no respiratory movements except gasping motions of nose. The pulse record appears as the side tube of the tracheal cannula is closed. Probably heart block occurs rather than slowing, as seen in *G*, a similar record taken after 5 minutes more of thorough ventilation. On this record a tambour was added (bottom) for manual signaling of nose movements. These appear to be reflexly associated with the heart blocks, preceding the latter by about 3 heart beats at first, then by 2. *H*, after thorough ventilation for 15 minutes more, the heart has recovered from block, and now slows gradually, but at a much earlier stage of asphyxia than normal. Movements of nose associated with gasping respiration in the normal animal start at about the stage that the normal respiration should start after apnea. The heart fails before the respiration. Middle trace, signal of nose movements. Time in seconds for all records.

between two respiratory phenomena not dissociated in the normal. While the last traces of respiratory movement were still being recorded, a slower apneustic or gasping movement was added to it (fig. 2, C), observable particularly in the movement of the nose, and the two sets of movements were not in phase. Even after air movements could no longer be recorded, nose movements could be observed. These were suppressed during artificial ventilation, and reappeared at an early stage of asphyxia, not with the periodicity of normal respiratory movements but with the frequency typical of terminal gasping (fig. 2, G and H). Since, as indicated by heart rate (see below), the animal was really not yet in the

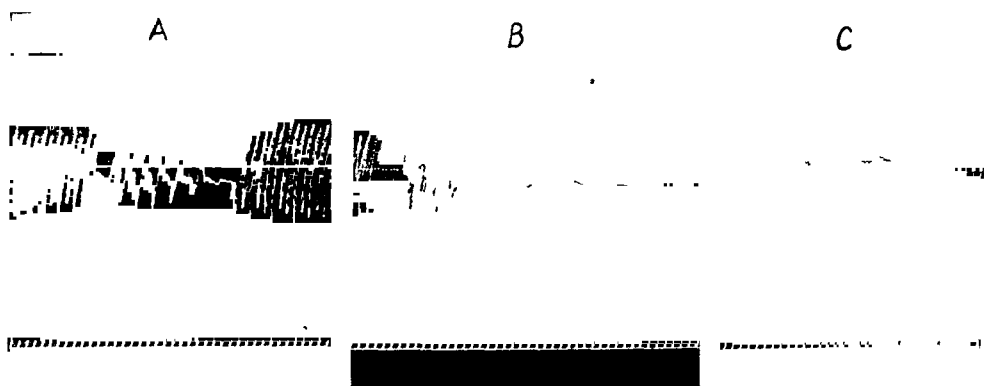


Fig 3. Record from tracheal cannula of rabbit prostrated 48 hours earlier by partial respiratory paralysis, 20 M.L.D. intravenous. Respiratory movements ceased after 24 hours, but previous to this could be maintained for over a minute after apnea at a time when too little air was moved to ventilate the lungs. Time in seconds. A, artificial ventilation stopped, tracheal side-tube closed, pulse record from lungs showing 3-1 block immediately after ventilation. B, a similar record, allowed to run until only an occasional heart-beat was recorded. The apparent slowing of the heart is really heart block, for a very slight pulse can be read on the record between the slow beats, with the original rate. C, an attempt was made to release the block by over-ventilation, which reduced the force of the beat but only decreased the block ratio to 6-1.

stage of extreme oxygen want, it is believed that this type of respiration suggests direct effect of the toxin on the respiratory center, as expressed in both depression and dissociation.

2. *Failure of the heart.* In similar experiments the heart rate could be followed during the period between apnea and asphyxia. Some of these animals have died suddenly under direct observation with every evidence of adequate artificial ventilation. Realizing that the rabbit heart is rather liable to failure under experimental conditions, some of these deaths we still believe we can assign to heart impairment induced directly by the toxin.

The normal rabbit heart, under ether, with the chest opened, following

periods of artificial ventilation, may beat in the ensuing asphyxial period for as much as $2\frac{1}{2}$ minutes after respiratory efforts have ceased. Our experimental animals, completely paralyzed by not too large doses of toxin, sometimes nearly duplicate this performance. Usually, however, this is not the case; the heart slows sooner than the normal during asphyxia after apnea, and may almost stop even before respiratory efforts have ceased (fig. 2, F, G, H). The only two animals which have died during actual observation of the heart record have died during asphyxial periods, not by sudden cessation of the normal frequency, but due to the persistence of the operator in recording the one more final beat which failed to eventuate, before turning on the air. In both cases, however, earlier in the experiment the heart had not slowed so much, nor—obviously—failed to recover at the same period after ventilation had stopped.

We feel that, while no one experiment is conclusive, the accumulated evidence suggests a direct effect of very large doses of toxin on the heart, but no appreciable effect of doses just sufficient to cause respiratory paralysis; that is, the heart is less sensitive to the toxin than is the skeletal motor apparatus. Stimulation of the vagus peripherally in a few experiments produced a fair slowing of the heart even in advanced stages of intoxication.

3. *Nerve-muscle failure.* After cessation of respiratory movements, and when artificial ventilation will no longer restore them by oxygenating the blood supply, stimulation of the peripheral stump of the cut phrenic nerve also fails to cause contraction of the diaphragm. Stimulation of the sciatic of a paralyzed animal also has no effect on muscles innervated. Direct stimulation of the muscles causes contraction, and the contraction is conducted along the fibers of a parallel fibered muscle such as the sartorius. We have not investigated to what degree such a contraction is of normal strength or time relations, but the facts are sufficient to identify a curare-like block at the myoneural junction, provided the nerves involved are also functional. Excised nerves from such animals give normal action potentials as recorded on the oscillograph. We thus substantiate the conclusion of Edmunds *et al.* that the myoneural junction is the critical structure whose failure causes death in botulism.

These experiments do not indicate whether the partial recovery from paralysis of asphyxiated animals due to artificial ventilation is a recovery of the myoneural junction or a recovery of the centers. The change in frequency of respiration on readministering air in the previous type of experiment is only conclusive in showing that the intoxicated center reacts qualitatively at least as the normal does. Since the myoneural junction of the normal warm-blooded animal is also affected by asphyxia, this recovery in the intoxicated animal may be viewed as the normal process of recovery acting in an impaired structure.

4. *The phrenic neurogram.* Animals which had received large doses of toxin, after paralysis of respiration, were given artificial respiration by tracheal cannula under ether, the chest opened, the phrenic nerve cut next to the diaphragm and dissected free up to the level of the base of the heart. The end was crushed and mounted on the amplifier grid electrode, a ground electrode was attached 1 cm. or more away, and a second

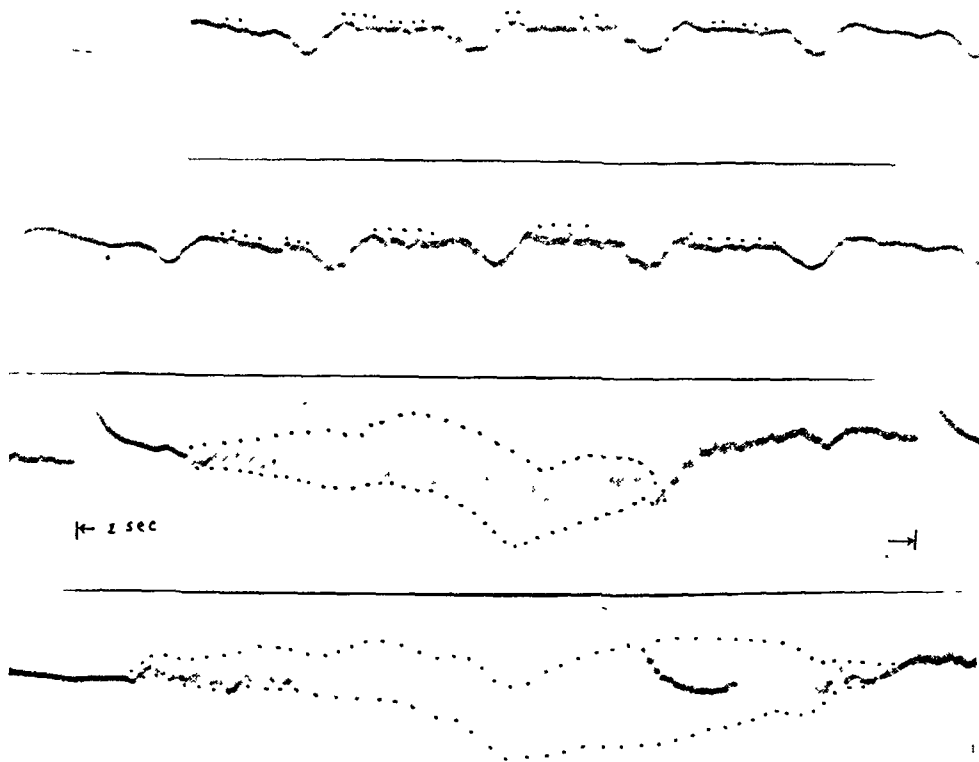


Fig. 4. Phrenic neurograms of normal rabbit, 1st, 3rd, 16th and 31st inspirations after apnea. In the first two records the heart beat is recorded due to mechanical jerking of the nerve on the electrodes. Heart rate 4 per second. In the third strip the time between two upward breaks in base line is 1 second. The last record shows the maximum amplitude of the series, but duration of discharge was still more prolonged in subsequent inspirations. Dotted lines trace the faint outlines of the amplitude.

ground made contact with the nerve near its attachment. Ventilation was applied until the phrenic neurogram as observed on the oscillograph was absent, that is, until apnea was induced. A camera was then started which drew a 60 mm. wide strip of bromide recording paper across the face of a second oscillograph tube, operating in parallel to the observation tube from the same amplifier. After ventilation was stopped, sample respirations were recorded until either the heart slowed dangerously or the

respiratory responses grew brief and weak. Normal animals were similarly recorded as controls. The lungs were collapsed throughout the periods between ventilations.

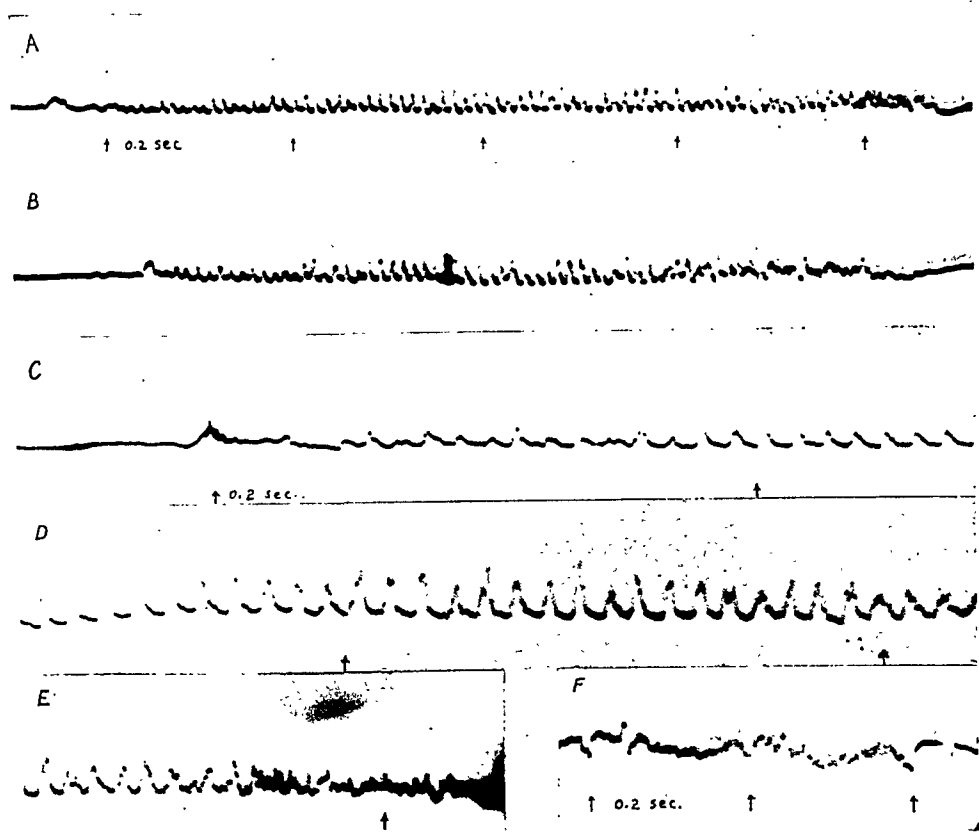


Fig. 5. Phrenic neurograms of intoxicated rabbit after respiratory paralysis, 20 M.L.D. intravenous. First strip, 8th inspiration, second 15th after apnea, increase in amplitude but not in duration. Arrows mark $\frac{1}{2}$ second, recorded by short shocks too faint to reproduce. Strips 3, 4, and first half of 5, the 10th inspiration with faster movement of paper to show striking synchronism of volleys, which, however, is probably not assignable to the toxin. Time in $\frac{1}{2}$ second, records continuous one with the next. Note breaking of synchronization at end of inspiration. Last record, 15th discharge from a different rabbit, lasting $\frac{1}{2}$ second, maximum duration of which this animal was capable, 20 M.L.D. intravenous. Heart beats recorded due to mechanical movement of nerve on electrodes, 4 per second. Shortening of duration of discharge and earlier failure under asphyxia presumably assignable to toxic action on center.

In the normal animal under light anesthetic, thirty to fifty respirations can be recorded between apnea and asphyxia, a period lasting up to two minutes. Each discharge down the phrenic consists of a series of volleys more or less discrete (Gasser, 1928), starting and ending at low amplitude

but of approximately constant frequency when distinct enough to measure. Successive discharges may increase in duration and amplitude up to at least the thirtieth (fig. 4), then decrease in amplitude while still increasing in duration, and finally decrease in both amplitude and duration to cessation. The heart may or may not have slowed before respiration ceases, but may continue to beat for at least 2 minutes longer.

In some animals, after complete paralysis of the respiratory musculature, the phrenic discharge is normal, and the changes in its character during asphyxia follow the normal course (fig. 5, A-E). In others, in which the heart action has been still good, the duration of the separate respiratory discharges has been distinctly briefer than normal, and the time of cessation during asphyxia also less (fig. 5, F). The appearance is that of a center more easily fatigued, and therefore less able to withstand withdrawal of oxygen. This is a well-known effect of curare on the myoneuronal junction, and presumably connotes here a depression of central nerve synapses, but only, it is obvious, at concentrations much above the threshold for muscle. It happens that our most clearly reproducible records are those from intoxicated animals, and these are clear because of the close synchronization of the discharges in separate fibers taking part in the volley (fig. 5, A to E). This synchronization cannot be taken as an effect of the toxin, however, since the phenomenon is observed also in the normal animal (Gasser) and these particular animals happen not to have shown any undue signs of depression assignable to the toxin; their discharges approached the normal in duration and number. It can be concluded that depression in peripheral nerve is not involved in any toxic action exhibited by the intact animal, and that the gradual and progressive depression of respiratory function indicated in the kymograph records of the longer lasting experiments on intact animals involves in part a central depression such as is found here.

5. *Peripheral nerve.* In treating excised tissues with toxin, even more than in treating the animal as a whole, the effects of substances other than toxin in the material containing it become important. For instance, nerve is notoriously responsive to changes in certain salt concentrations, and it could be anticipated that to obtain any effect the dosage of toxin would have to be extreme. Since the toxin can be inactivated by heating, control nerves were exposed to heated toxin. The straight solution heated or not had a depressant action. In toxin diluted with equal parts of Tyrode solution the nerves were gradually depressed, but recovered promptly in Tyrode to a state not far different from that when kept in Tyrode alone. A pair of vagus nerves from an animal receiving 40 lethal doses were removed after complete paralysis of the animal and kept for $5\frac{1}{2}$ hours in the Tyrode-toxin mixture containing 10,000 mouse M.L.D.'s

per cc., at 6°C. They were tested initially, after 3½ hours, and after 5½ hours, one each first in the solutions of active and inactive toxin and then after a brief soaking in Tyrode. In the toxin solutions they were depressed, but not to extinction of action currents. In Tyrode they returned promptly to a reasonable approximation of their original activity, the one from the active toxin being in as responsive a state as the one from the inactivated solution. This and other modifications of the procedure showed no effect whatever attributable to the toxin in peripheral nerve.

CONCLUSIONS

Rabbits intoxicated with varying dosages of botulinus toxin have been maintained in an artificial respiratory apparatus after complete paralysis, and their condition tested. Death without artificial interference ensues not when paralysis is complete but when the weakening of some critical musculature prevents the tidal air from ventilating the alveoli. At this stage of paralysis, if oxygen is supplied artificially, skeletal muscular activity is by no means abolished.

The immediate cause of death in rabbits is usually the sealing of the external nares by dried mucus, at a stage when the nasal musculature is just weakened sufficiently to prevent reflex opening. This circumstance may account in part, but not wholly, for the variability of the M.L.D. for rabbits.

At the time of first prostration, which, if aid is not administered, is followed very promptly by suffocation, animals can still respire sufficiently to maintain life if the air passages are kept open; and if artificial ventilation is supplied, respiratory movements may be observed for many hours after prostration, the time depending on the dosage.

Death without artificial ventilation always occurs from paralysis of the myoneural junction by a curare-like action of the toxin, or by heart failure, probably heart block, of which partial asphyxia is the immediate cause. With massive doses, and long after paralysis of skeletal musculature, partial or complete heart block and depression of the respiratory center under artificial ventilation can probably be assigned to direct toxic action on these structures. Under the action of toxin in large doses, such effects are induced by a degree of asphyxia decidedly less than that required in the normal animal, or occur in spite of normally adequate ventilation.

The specificity of the toxin for the myoneural junction is at least as great as that of other pharmacological agents for their characteristic sites of action.

No effect of toxin in any concentration that could be employed, in the body or out, could be observed on peripheral nerve.

REFERENCES

- BRONFENBRENNER, J. J. AND H. WEISS. J. A. M. A. **76**: 1741, 1921.
Proc. Soc. Exper. Biol. and Med. **19**: 296, 1922.
J. Exper. Med. **39**: 517, 1924.
- EDMUNDS, C. W. AND P. H. LONG. J. A. M. A. **81**: 542, 1923.
- EDMUNDS, C. W. AND A. F. KEIFER. J. A. M. A. **83**: 495, 1924.
- GASSER, H. S. This Journal **85**: 869, 1928.

THE TETANY OF OESTRUS IN THE PARATHYROIDECTOMIZED DOG

EVERETT I. EVANS, S. SZUREK AND R. KERN

From the Department of Physiology, University of Chicago

Received for publication May 29, 1936

It has been known for some time that some women after thyroidectomy suffer attacks of tetany at each menstrual period, but it remained for Luckhardt and Blumenstock (1922) to call attention to the rôle of the parathyroids in these phenomena. These workers showed that female dogs parathyroidectomized some months before oestrus, treated with intravenous Ringer's solution immediately after the operation to control the tetany, again showed symptoms characteristic of parathyroid deficiency at the onset of the next oestrous cycle (anorexia, hyperpnea, retching, etc.). Luckhardt and Blumenstock were unable to explain the recurrence of severe tetany during the oestrous cycle, months after the operation, but suggested that the symptoms pointed to a general paresis of the sympathetic system. The earlier observations were confirmed by Dragstedt, Phillips, and Sudan (1925), who likewise were unable to explain the cause of this tetany.

While we were making observations on the blood chemistry of parathyroidectomized dogs in the latent survival period, an opportunity was afforded to determine several inorganic ions before and during oestrus in the parathyroidectomized dogs in our series of experiments. We hoped to throw some light on the problem by determining whether or not oestrus caused a further lowering of serum calcium which might explain the tetany in oestrus of the parathyroidectomized dog.

EXPERIMENTAL. Healthy normal female dogs were selected from the animal colony and after an adequate control period, a bilateral thyroparathyroidectomy was performed under ether-morphine anesthesia. The thyroids were carefully removed and the thyroid vessels ligated as close to the carotid artery as possible. In all the dogs the characteristic symptom complex of parathyroid deficiency developed within 48 to 60 hours after the operation if the calcium was removed from the diet.

Calcium lactate (Luckhardt and Goldberg, 1923), and calcium gluconate (Szurek, 1929) were given in doses of 1.5 and 3.0 grams per kilo, respectively, each day in the food or by stomach tube. This procedure was carried out for 30 to 60 days and by this means postoperative tetany

was controlled. The diet was bread and meat, care being taken not to give too much of the latter.

When all symptoms of tetany had disappeared, these dogs were considered to be in the "survival" period. Blood samples were taken at convenient intervals and the animals carefully observed for any signs of tetany. If any signs of tetany developed they were relieved by calcium therapy. On no occasion was a blood sample taken until 12 to 18 hours had elapsed following the dog's last meal.

Calcium, phosphorus, sodium, potassium and chlorides were determined in duplicate on the sera of these animals. The methods used were the following:

Chlorides—Wilson and Ball (1922)
Calcium—Kramer and Tisdall (1921)
Phosphorus—Fiske and Subbarow (1925)
Sodium—Kramer and Gittleman (1924)
Potassium—Kramer and Tisdall (1921)

Blood chemistry studies were also made on three normal dogs on a similar diet for some time before, during, and after oestrus. In these animals, calcium, phosphorus, pH and CO₂ were determined on the serum and data of one of these dogs as shown in table 1 to serve as a comparison with those data obtained in the parathyroidectomized series.

RESULTS AND DISCUSSION. In table 1 are given data on four normal female dogs and, for sake of space economy, the condensed protocols are shown for only three of our six parathyroidectomized female dogs that came into oestrus during the latent tetany period.

These data illustrate that in the normal dog there is no significant change in the values for blood calcium, phosphorus, pH and CO₂ during anoestrus, proestrus, oestrus, or metoestrus. The values for the inorganic ions and pH and CO₂ are quite within the normal range.

The data shown for dog 6 are characteristic of our findings for the parathyroidectomized female dog in oestrus in the latent tetany period. During anoestrus, her blood picture shows a remarkably low serum calcium. There was no marked change in proestrus. On the second day of oestrus the blood picture was practically the same, but mild tetany was evident. No change in her behaviour was noted on the seventh day of metoestrus, but the serum calcium was found to be slightly raised. This parathyroidectomized dog, then, illustrates a case of low serum calcium during oestrus, with a rise in metoestrus, and only a very mild tetany.

Dog 5, on the other hand, suffered severe attacks of tetany in oestrus, although her blood values were close to the normal. On the third day of oestrus, attempts were made to service the dog after a blood sample

TABLE 1

DATE	Ca	P	Na	K	Cl	pH (20°C)	CO ₂	REMARKS
(a) Normal								
7-24-29	10.6	3.9	326	22.9	627			Dog 1 Definitely in oestrus
7-31-29	10.8	3.8	339	23.1	649			Metoestrum. Will not accept male
7-18-29	12.8	4.8	326	25.3				Dog 2. Accepts male
7-19-29	12.6	4.6	328	25.3	668			Oestrus 1st day metoestrus
1-10-33	11.44	5.7				7.56	52.7	Dog 3 Anoestrus
1-26-33	12.20	5.3				7.51	58.4	2nd day proestrus
1-31-33	12.88	4.6				7.53	57.2	1st day oestrus
2- 5-33	12.63	4.5				7.56	55.6	6th day oestrus
2-12-33	12.60	4.5				7.64	57.2	Metoestrus
3- 5-33	11.70	3.6				7.54	56.2	Dog 4 Anoestrus
3-11-33	11.62	3.7				7.56	55.2	3rd day proestrus
3-15-33	11.84	3.8				7.59	57.9	1st day oestrus
3-19-33	11.78	3.6				7.55	54.1	5th day oestrus
3-28-33	11.54	3.8				7.57	55.8	Metoestrus
(b) Parathyroidectomized								
12-18-28	7.8	4.5						Dog 5 Parathyroidectomy operation 10-27-28. Last tetany 11-26-28
1-21-29	7.4	5.7	414	22.0				No tetany
5-13-29	8.7	6.3	322	24.0	661			No tetany
5-27-29	9.8	6.0	348	21.6	650			No tetany Dog definitely in oestrus.
6- 5-29	7.4	6.5	380	20.3	649			Coitus and tetany.
6-25-29	11.3	5.6	391	21.9	668			No tetany. Metoestrus
7-26-29	6.5	6.4	315	22.9	628			No tetany. Dog pregnant
8-25-29								No tetany Dog aborted 13 cm. dead fetus. No tetany
5-16-29	5.9	6.3			668			Dog 6 Parathyroidectomy operation 4-29-29
5-21-29	5.5	5.8	315	24.5	694			No tetany
5-23-29	5.1	4.6	344	22.1	682			No tetany No tetany. Proestrus

TABLE 1—*Concluded*

DATE	Ca	P	Na	K	Cl	pH (20°C)	CO ₂	REMARKS
(b) Parathyroidectomized— <i>Concluded</i>								
5-27-29	4.8	4.0	298	20.4	658			Definitely in oestrus Few tremors, but no marked signs of tetany. Gave no medication
6- 8-29	7.2	5.6	367	21.5	670			External genitalia still enlarged Metooestrus
6-23-29								Animal appears ill Anorexia. Decision to give calcium by stomach tube Animal developed diaphragmatic spasm and died in half hour of respiratory failure
5-21-29	11.7	4.5	338	26.1				<i>Dog 8</i> Parathyroidectomy 1-10-29. Last tetany 2-7-29 2nd day prooestrus
5-27-29	5.4	6.0	351	34.0	615			3rd day oestrus No tetany but anorexia
5-29-29	5.8	6.1	342	25.2	630			5th day oestrus No tetany
6- 3-29	5.6	4.9	361	24.6	662			Metooestrus
6-11-29	7.0	5.4	356		629			Had developed "snuffles." Dead 6-15-29 in depression

had been taken. Successful copulation with a parathyroidectomized male was observed, after which the female dog developed a severe attack of tetany. (Blood calcium 9.8, phosphorus 6.0.) Calcium gluconate was given by intravenous injection and the dog recovered. By 6-25-29 it was evident the dog was pregnant and to our surprise the blood sample showed a normal serum calcium. No signs of tetany were present thereafter, despite the lowered serum calcium on 7-26-29. During an experiment on vaginal motility on 8-25-29 the dog aborted a 13 cm. fetus; no tetany was observed before or after this abortion.

Dog 7 (not considered in table 1) died on the second day of oestrus (with serum calcium 6.1, phosphorus 8.6) after a violent attack of tetany. A week before, on the first day of prooestrus, the serum calcium was 8.0 and phosphorus 4.9. It would appear in this instance that the drop in serum calcium and rise in phosphorus were important factors in this

tetany. However, there is the case of dog 8 (table 1) who had on the third day of oestrus a serum calcium 5.4, phosphorus 6.0, a change from the prooestrous figures of calcium 11.7, phosphorus 6.0 and yet no tetany occurred during oestrus.

Mathieu and Barnes (1933) demonstrated that when theelol or theelin is administered to parathyroidectomized female dogs, a fall in serum calcium occurs, the lowered level persisting throughout the injection period. They found no correlation between the drop in serum calcium and tetany in "artificial" oestrus and observed only one attack of tetany with this drop in serum calcium. Their study indicates that in parathyroidectomized female dogs, a low serum calcium may be further lowered by female sex hormone without precipitating tetany.

In conclusion, then, it may be said that our data indicate that in the parathyroidectomized female dog in natural oestrus in the latent tetany period, tetany can occur with either a further drop in serum calcium or when the serum calcium level is maintained at the anoestrous level; we would conclude, then, that the occurrence of tetany in oestrus in the parathyroidectomized dog is not dependent upon a further lowering of the possibly already low serum calcium. Changes in serum phosphorus, sodium, potassium, or chlorides (and probably pH and CO_2) are probably not involved in the severe tetany of oestrus in parathyroidectomized female dogs.

Those who have closely observed unoperated female dogs in oestrus are well aware that the bitch at this time is often highly excited and restless. The neuromuscular balance of the parathyroidectomized dog is easily upset and it is altogether possible that the greatly increased nervous discharges during oestrus would so upset this balance as to throw the animal into tetany. Our impression is that the cause of this tetany of oestrus in parathyroidectomized dogs is linked more closely to an instability of the nervous system rather than to changes in ionic balance in the blood stream at oestrus.

SUMMARY

There is no apparent change in the blood values for several inorganic ions and pH and CO_2 of normal female dogs during the oestrous cycle. Tetany can occur in the parathyroidectomized female dog coming into natural oestrus in the latent tetany period with either a comparatively normal or low serum calcium. It appears that other physiological changes are responsible for this type of parathyroid tetany.

The authors gratefully acknowledge the advice and assistance of Dr. Arno B. Luckhardt in these experiments.

REFERENCES

- DRAGSTEDT, PHILLIPS AND SUDAN. This Journal 65: 503, 1923.
FISKE AND SUBBAROW. J. Biol. Chem. 66: 375, 1925.
KRAMER AND TISDALL. Ibid. 47: 475, 1921.
KRAMER AND GITTLEMAN. Ibid. 62: 353, 1924.
KRAMER AND TISDALL. Ibid. 46: 339, 1921.
LUCKHARDT AND BLUMENSTOCK. Science 56: 257, 1922.
SZUREK, S. Proc. Soc. Exper. Biol. Med. 26: 773, 1929.
LUCKHARDT AND GOLDBERG. J. A. M. A. 80: 79, 1923.
WILSON AND BALL. J. Biol. Chem. 79: 221, 1922.
MATHIEU AND BARNES. This Journal 105: 173, 1933.

CARDIOVASCULAR REACTIONS INDUCED BY ELECTRICAL STIMULATION OF THE CEREBRAL CORTEX^{1,2}

EBBE C. HOFF AND HAROLD D. GREEN

From the Laboratory of Physiology, Yale University School of Medicine

Received for publication June 1, 1936

The existence of cortical influence upon the cardiovascular system has been indicated in part by observations of heart rate and blood pressure changes in patients with known cerebral lesions (Minkowski, 1; Popper, 2), and in subjects capable of voluntary cardiac acceleration (Taylor and Cameron, 3). Such influence has been further suggested by clinical observations of localized disturbances of vasomotor activity in cases of hemiplegia beginning with work of Chevallier in 1867 (4), to which attention has recently been called by Fulton (5); and in studies on animals with experimental lesions of the cortex. (See Pinkston, Bard and Rioch (6), and Kennard (7).) Additional evidence has also been adduced from the results of electrical stimulation of the surface of the cerebral hemispheres. Studies of the latter type began with the discovery by Schiff (8) that the heart rate could be altered, and by Danilewsky (9) who pointed out that the blood pressure could also be changed. In spite of the fact that these findings have been repeated several times, Bard (10) has felt "that many of the effects which were produced are to be accounted for on the basis of spread of strong stimulating currents to subcortical regions or to production of cortical epilepsy in which a widespread and unphysiologic discharge occurs." Thus, although clinical and physiological literature contains the implication that the cerebral cortex plays a part in the regulation of cardiovascular functions, nevertheless such cortical influence has neither been completely established nor universally accepted. The investigations to be reported in the present paper are an attempt to elucidate this relationship in controlled stimulation experiments.

METHODS. This report is based on a study of 40 cats, 18 monkeys (*Macaca mulatta*) and 1 chimpanzee anesthetized with ether. The cerebral cortex was stimulated by a Harvard inductorium, a thyratron tube discharging a condenser through a

¹ This investigation was aided by a grant from the Research Funds, Yale University School of Medicine.

² Preliminary reports of these experiments were communicated to the Yale Medical Society, November 13, 1935 (*Yale J. Biol. Med.* 8:201, 1935), and to the American Physiological Society, March 26, 1936. (*This Journal*, Proc. 1936.)

loosely coupled step-up transformer or the standard 110 volt 60 cycle alternating current reduced to the proper voltage by means of a potentiometer. In most cases simple bipolar electrodes were used with the tips separated approximately one millimeter. Blood pressure was recorded in most instances from the femoral artery by either a mercury manometer or a Wiggers optical manometer. Pupillary changes were observed. In many experiments a slow intravenous infusion of saline or Locke's solution was maintained. In a few cases epinephrine was added to this perfusion fluid in the proportion of 2 to 4 drops of a 1:1,000 solution to 250 cc. of perfusion fluid. Since a finer adjustment of stimulus intensity was required to differentiate cardiovascular responses from skeletal muscular movement than was usually convenient, the expedient was adopted in the later experiments of injecting sufficient curare to prevent contractions of the skeletal muscles.

RESULTS. 1. *Changes in blood pressure.* In cats and monkeys anesthetized with ether stimulation of the motor region of the cortex regularly produced a rise in blood pressure after a latent period of 1.5 to 10 seconds followed by a return to normal beginning approximately the same number of seconds after the cessation of stimulation. With a prolonged stimulation the pressure reached a peak after 10 to 20 seconds and then began to decline slowly, the rate of fall usually being accelerated by cessation of stimulation. Associated with the rise in pressure, there was often an acceleration of the heart of 5 to 10 per cent, while a slowing of as much as 50 per cent often occurred during the post-stimulatory decline of arterial pressure. Records illustrating rise of pressure are reproduced in figures 2 to 6. In all records the points of stimulation are indicated by a numbered dot on the diagram accompanying the record.

In one chimpanzee, anesthetized with ether and in the latter part of the experiment curarized to prevent muscular movements, a rise of pressure amounting to 10 to 20 mm. Hg has been elicited by stimulating the motor and premotor cortex in the region adjacent to the superior precentral sulcus and the posterior part of the superior frontal sulcus using the A.C. 60-cycle current at a strength of 3 to 4 volts. These pressor responses were usually associated with muscular activity and occasionally with epileptiform contractions. However, the rise in pressure usually preceded the muscular activity, beginning after a latent period of 3.5 to 5.0 seconds and commencing to decline within 4.0 seconds after cessation of the stimulus. The return to pre-stimulation level was complete in 25 seconds. The rises in blood pressure were greater after abolition of muscular responses with curare and were evoked with a lower current strength than before curare was administered. This facilitation of pressure rise from cortical stimulation following the administration of curare has also been observed in experiments on cats and monkeys.

Various points on the parietal cortex were stimulated in 11 cats anesthetized with ether. No alteration of blood pressure occurred in 5 of these, but in 6 of them a decline of pressure of 10 to 15 mm. Hg followed

stimulation of appropriate points in this area. In many instances of maintained excitation this fall was succeeded by a rise. In the monkey a decline of pressure was not as commonly observed as in the cat but the blood pressure was sometimes diminished during stimulation of the marginal gyrus and a small area of the cortex near the anterior tip of the superior precentral sulcus (fig. 1, C and D). A typical fall of pressure in a cat is illustrated in figure 2, record 53.

2. *Changes of heart rate.* The cortex on and adjacent to the gyrus proreus was stimulated in 5 cats deprived of both stellate ganglia and both adrenal glands. No change in heart rate was observed in two of them, but in 3 of these animals the heart was slowed by as much as 50 per cent. A similar slowing of the heart was obtained from a point 1 cm. anterior to the superior precentral sulcus in one monkey on which the same operative procedures had been performed. In all cases the slowing was asso-

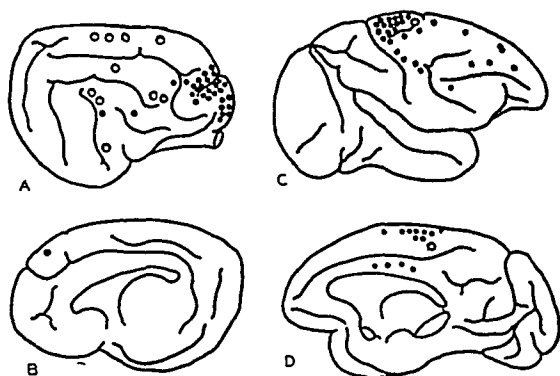


Fig. 1. Diagrams of the pressor and depressor points. A, lateral and B, medial surface of the cat's cortex; C, lateral and D, medial surface of the monkey's cortex. Solid dots—rise of pressure; circles—fall of pressure.

ciated with either no change or a fall of pressure. Since these changes in heart rate have been only occasionally observed, it has not been possible to determine definitely whether they are the result of vagal stimulation or sympathetic inhibition, but, since they were obtained in stellatectomized, adrenalectomized animals, the slowing would appear to have been primarily a vagal effect.

3. *Other autonomic phenomena.* In both cats and monkeys, dilatation of the pupils, and in cats, retraction of the nictitating membranes occurred simultaneously with the rise in pressure obtained from the gyrus proreus and the anterior sigmoid gyrus (cat) and the area just above the superior precentral sulcus (monkeys). The pupils and nictitating membranes returned to the basal state as the pressure fell. Gastric responses were not studied.

In one monkey (C.S. 34) distinct elevation of the hair on the dorsum

of the shoulders and upper back was obtained from stimulation of points on the cingulate gyrus corresponding to the four dots placed in this area in figure 1 D.

4. *Cortical areas from which cardiovascular responses were obtained.* In the cat under ether anesthesia the vasopressor responses, indicated by solid dots in figure 1, A and B, were obtained most regularly from the posterior and anterior sigmoid gyri and the adjacent part of the gyrus proneus. Occasional pressor points were also located on the anterior portions of the suprasylvian and ectosylvian gyri and on the anterior lip of the sylvian fissure. As figure 1 indicates, elevations of pressure were also recorded during stimulation of a point on the marginal gyrus on the medial surface of the hemisphere. Depressor points, indicated by circles, were consistently located in the cat on gyrus lateralis medius, gyrus suprasylvius medius, gyrus ectosylvius medius and gyrus sylvius.

Rises in pressure were most readily obtained in the monkey anesthetized with ether from the cortical areas adjacent to the superior precentral sulcus (fig. 1, C and D). Vasopressor responses also occurred during stimulation of the trunk and arm areas and the superior, middle, and

Fig. 2. A consecutive series of records illustrating the close proximity of pressor and depressor points in a cat. Male, weight 3.0 kgm., ether anesthesia, curare 45 mgm., Harvard inductorium 8.5 cm., artificial respiration, saline infusion. Record 52, a rise in pressure obtained from point 28 just in front of the anterior tip of the lateral sulcus; record 53, a fall of pressure due to stimulation of point 27 lying on gyrus supra sylvius anterior 3 mm. lateral to point 28; record 54, a rise of pressure from stimulation of point 26 on the gyrus suprasylvius anterior, posterior to point 27, and approximately 3 mm. from points 27 and 28; record 55, stimulation of point 27. Experiment C. S. 23.

Fig. 3. Consecutive records illustrating absence of current spread in monkey. Female, *Macaca mulatta*, weight 3.5 kgm., ether anesthesia, curare 10.5 mgm., Harvard inductorium 12 cm. angle 20 degrees from horizontal, artificial respiration, saline infusion. Record 29, stimulation of point 9 lying on left leg area 1 mm. anterior to central sulcus; record 28, stimulation of point 18 1 mm. posterior to the central sulcus and not more than 3 mm. distant from point 9. Experiment C. S. 32.

Fig. 4. Records demonstrating that blood pressure responses are abolished by local anesthesia of the cat's cortex to a depth of 3 mm. Female, weight 2.9 kgm., ether anesthesia, curare 20 mgm., Harvard inductorium 8.5 cm., artificial respiration, saline infusion. Records 12 and 13, stimulation of points 5L and 5R on the left and right posterior sigmoid gyri. Immediately after the record 13, a 2 per cent solution of Nupercaine hydrochloride N.N.R. was applied by means of a small pledget of filter paper to the right cortex in the region corresponding to point 5R. Records 30 and 31, stimulation of points 5L and 5R 32 minutes after beginning the application of Nupercaine to 5R. Record 32, stimulation of point 5R with the electrodes pushed 3 mm. into the cortex.

Fig. 5. Pressure rise unaccompanied by skeletal muscular movement in a non-curarized cat. Male, weight 3.0 kgm., ether anesthesia, Harvard inductorium 7.5 cm. Experiment C. S. 16-10-3R.

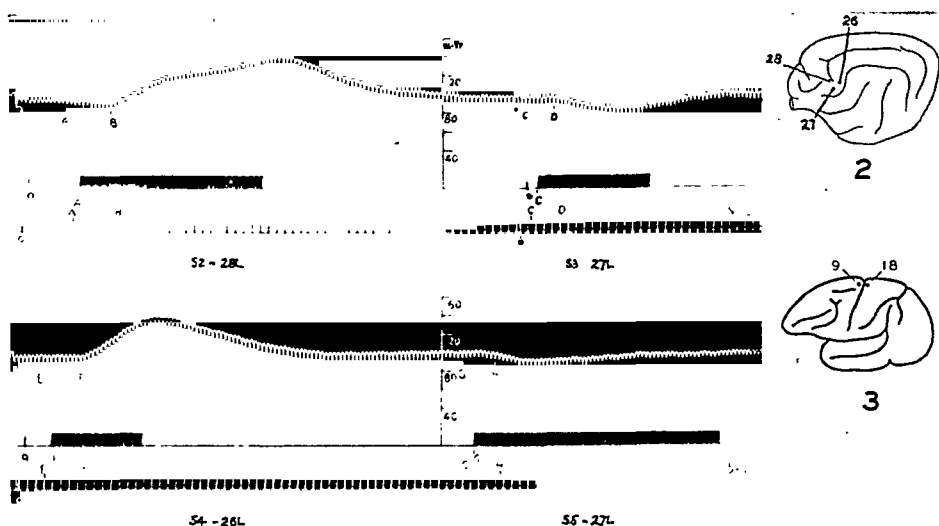


FIG. 2

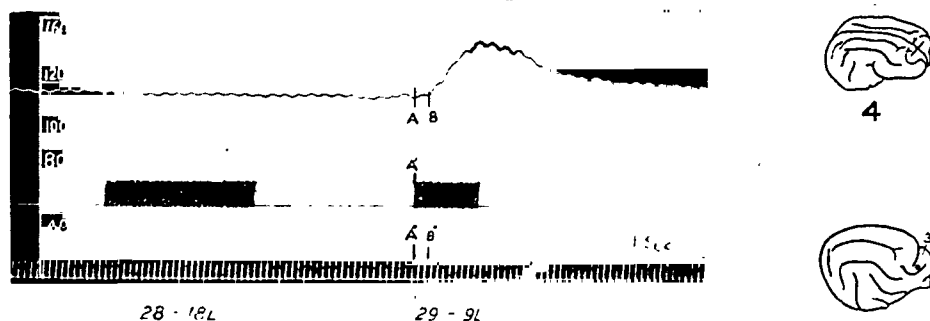


FIG. 3

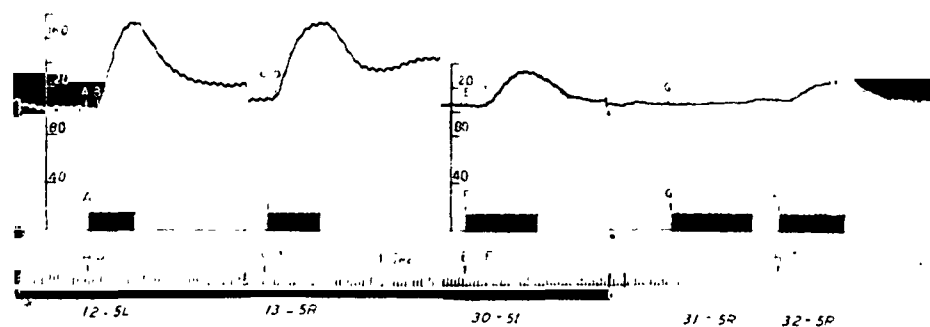


FIG. 4

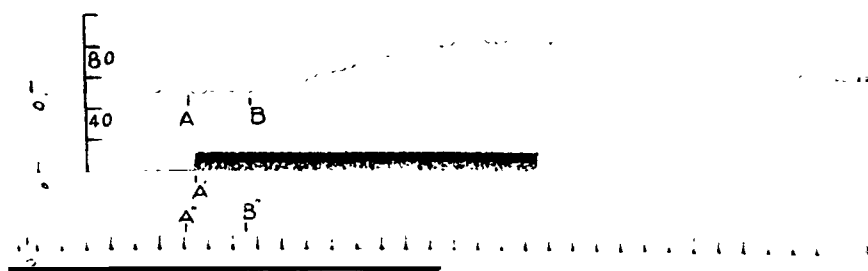


FIG. 5

Figs. 2-5

inferior frontal gyri. Points on the marginal and cingulate gyri on the medial surface, anterior to the central sulcus also gave pressor responses. The areas giving a fall of pressure have already been indicated.

Although other surfaces of the cortex have been explored, no additional points have been found which produce vasomotor changes. Thus in the monkey, the postcentral gyrus in particular as well as many points on the parietal lobe, the occipital lobe, and the upper half of the temporal lobe all failed to give vasomotor responses. The orbital surfaces of the

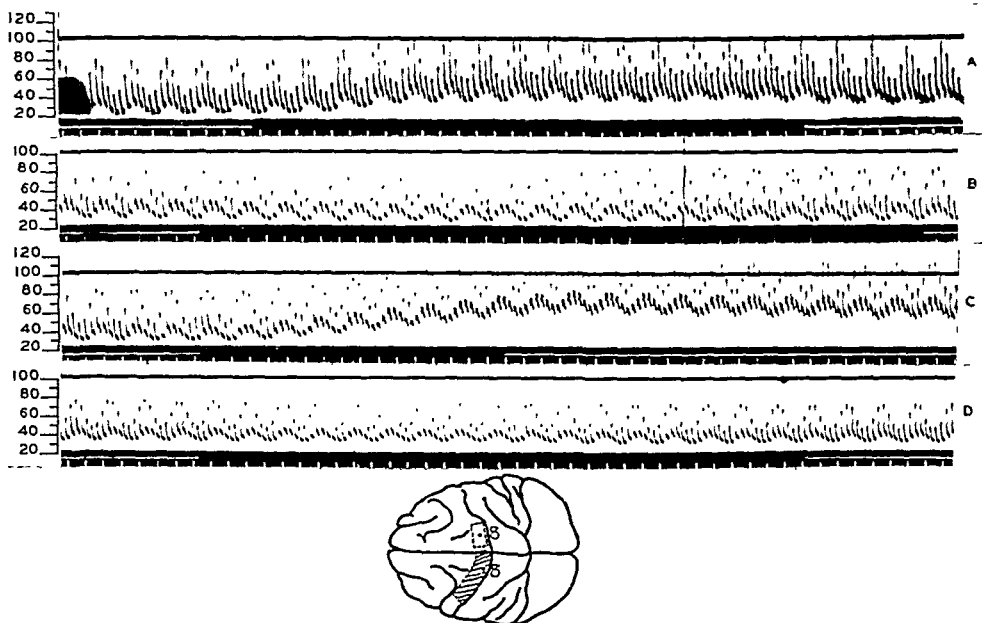


Fig. 6. Demonstration of the localization of the stimulated cells within the cortex of the monkey. Female, *Macaca mulatta*, weight 4.65 kgm., left area 4 (Vogt) removed 109 days before, ether anesthesia, curare 18 mgm., thyatron stimulator—60 cycles per second 150 peak volts, artificial respiration, A-28—normal response from point 8R on right cortex; B-30—absence of response from point 8R after undercutting; C-31—response from white matter exposed by removal of a block of cortex including point 8R; D-32—absence of response from point 8L in white matter exposed by previous ablation of left cortex. Experiment C. S. 42.

hemispheres were not explored nor were the medial surfaces with the exception of the point to which reference has already been made.

An examination of microscopical sections of the responsive areas of the cat's cortex has demonstrated that vasomotor responses were obtained from areas in which large pyramidal cells were present (areas B, E and F of Langworthy, 11, 12; and the motor cortex of Campbell, 13) as well as from areas in which they did not occur (area A of Langworthy and frontal cortex of Campbell).

5. Demonstration that responses are due to the stimulation of nerve elements

within the cortex. The above demonstrated cardiovascular responses resulting from the application of electrical currents to the cerebral cortex may have been due to the excitation of subcortical (e.g., hypothalamic) centers by spread of current from the points of stimulation, they may have occurred as a reflex response to stimulation of nerve fibers lying in the dura or accompanying the blood vessels of the cortex, or finally they may have been due to the activation of specific nerve elements within the cortex itself. That the first two possibilities cannot explain the changes in vascular tension and cardiac activity recorded, and that these findings may validly be considered the result of excitation of a nervous mechanism specifically localized within the cerebral cortex is suggested in the following three lines of evidence:

a. *Pressor and depressor points can frequently be discretely localized within 2 to 4 mm. of each other.* An experiment demonstrating this fact is illustrated in figure 2, taken from records obtained from the cat. Similar observations have been made in the monkey as illustrated in figure 3. In the latter example the path for the spread of electrical currents between the points was very short, although the points were separated some distance anatomically, while in the experiment illustrated in figure 2 the points were also very close together anatomically. Were the responses due to stimulation of nerves accompanying cortical blood vessels, such responses should always be the same and should be obtained from all parts of the cortex. It may be concluded, therefore, that the strength and manner of application of stimulation precluded spread of current of effective strength to adjacent cortical areas, to blood vessels, to dura or to subcortical structures.

b. *Local application of anesthetics to an excitable area of the cortex abolishes the responses from this point.* Records illustrating this phenomenon are reproduced in figure 4. Since the anesthetic abolished the response to stimulation of the cortex, and since the anesthesia extended only three millimeters into the depth of the hemisphere, it would seem reasonable to conclude that the responses must have been due to the stimulation of nerve elements lying within the outer three millimeters of the cortex. This experiment provides further evidence that current spread to subcortical centers plays no part in the vasomotor responses obtained.

c. *The response from a given point is abolished by undercutting this area of the cortex.* This phenomenon is illustrated in figure 6. That the rise of pressure was produced by stimulation of descending fibers from the cortex is shown by a comparison of record C with record D obtained while stimulating a corresponding point on the left hemisphere. This latter point lay on the white matter which had been exposed by removal of left area 4 sufficiently in advance to assure complete degeneration of the descending fibers. Since stimulation of point 8 on the left side pro-

duced no change of blood pressure it is presumable that the rise of pressure from stimulation of the white matter on the right side was due to the excitation of axones whose cell bodies lay within the cortex of area 4. Such axones while excitable on the right side (acute preparation), were degenerated on the left side (chronic preparation). Similar procedures have been carried out in the cat with the same results.

6. *Differentiation of vasomotor and somatic muscular responses.* François-Franck (14) and recently Mettler, Hamilton, Allen, Woodbury and Mettler (15) have stated that blood pressure changes from cortical stimulation are usually associated with an epileptiform seizure. François-Franck, in fact, never obtained cardiovascular responses from the cortex at a distance from the motor area except with stimuli which also provoked an epileptic attack. The rises in pressure accompanying these seizures were always of long duration, and since the blood pressure response to strong stimuli in the deeply curarized animals was entirely similar to that accompanying the epileptic attack in the non-curarized animals he suggested that while curare had abolished the motor manifestations of the seizure, the visceral component persisted (p. 188), and that the epileptic state was thus reduced to what he termed its organic or internal manifestations. As he stimulated nearer to the motor area simpler, briefer reactions appeared and with moderate stimuli applied to the motor cortex itself he obtained simple cardiovascular changes of brief duration unassociated with an epileptic state (p. 207).

Although we have obtained blood pressure changes associated with epileptiform discharges of cortical origin in our own experiments nevertheless it has been definitely possible to demonstrate specific alterations of blood pressure of brief duration unaccompanied by somatic responses. For example, in experiments 14 to 20 elevation of pressure was repeatedly obtained in cats lightly anesthetized with ether and without curare, using strengths of stimuli which produced no movement whatever. Figure 5 shows a typical record from one of these experiments. Furthermore we frequently noted as a result of cortical stimulation vigorous somatic movements of an epileptiform type which were unaccompanied by any alteration in blood pressure.

DISCUSSION. The observations reported in this paper conform in general to those of previous workers (see Weber, 16; Bechterew, 17; Dusser de Barenne and Kleinknecht, 18; Hunsicker and Spiegel, 19, and others quoted by these writers), who have all reported that either a rise of pressure or alterations of heart rate or both can be produced by cortical stimulation. That a fall in pressure can be consistently produced by cortical stimulation in the cat and dog was demonstrated by Dusser de Barenne and Kleinknecht (18). This finding has been confirmed in the cat under ether anesthesia in which, however, the points giving a fall in pressure

were restricted to the lateral, suprasylvian and ectosylvian gyri. It has not been possible under ether anesthesia to produce a consistent fall of pressure in the monkey.

In agreement with earlier observers the latency of the blood pressure responses to cortical stimulation has been found to be on the average 3.5 seconds. These short latent periods suggest that the cortical mechanism acts primarily through nervous channels. This suggestion is further supported by the fact that blood pressure rises still occurred after removal of the adrenal glands. Although in some of the experiments where the stimulation was continued for as long as 40 seconds the pressure reached a peak and began to decline on the average 16 seconds after the beginning of stimulation possibly as a result of fatigue, nevertheless it must be emphasized that in those experiments in which minimal stimuli were used the pressure always began to return toward normal within 2 to 4 seconds after cessation of stimulation and had usually reached the prestimulation level within 15 seconds. In instances in which excessive current strength was used the pressure did not fall at the end of the stimulation interval and frequently continued to rise further for 30 seconds to 2 to 3 minutes. This phenomenon is probably due to persistence of cortical activity, and may be referred to as after-response in the cardiovascular system.

In view of the evidence presented it must be concluded that there are nerve pathways from the cortex capable of altering the degree of vascular constriction and thus of causing a change of blood pressure. Whether these corticofugal pathways mediate their effect via the hypothalamus or whether they act directly on lower centers cannot at present be decided.

The location of the pressor and depressor points in cats confirms the general localization of these points by previous observers. In the monkey, vasopressor points have not previously been plotted. Although Schäfer (20) refers to some unpublished experiments in which he and Bradford observed vasodilatation resulting from stimulation of the cerebral cortex of monkeys in the region of the gyrus marginalis and gyrus fornicatus and vasoconstrictor effects from other regions of the cortex which were not specifically noted. These findings of vasopressor points in the motor area of the cortex have recently been confirmed in preliminary notes by Mettler *et al.* (15), and by Crouch and Thompson (21).

The epileptiform motor after-response which follows stimulation of the cortex with excessive strength of stimulation is well-known. That a visceral type of epileptiform after-response can also be initiated from the cortex has been demonstrated by certain experiments in which excessive strengths of stimuli, deliberately used, resulted in the persistence of a continued rise or alternate rise and fall of pressure lasting from 30 seconds to 2 or 3 minutes after cessation of stimulation. Since the visceral after-

responses have been obtained in etherized animals and also in animals in which all muscular response has been abolished by curare, these phenomena cannot be due to a delayed effect of muscular contraction. Furthermore when the strength of stimulation is reduced, a normal rise of pressure still occurs, but, on discontinuing the stimulation, pressure does not remain elevated but rapidly returns to the pre-stimulation level. Therefore it seems probable that the after-response in the cardiovascular system is due to the abnormal persistence of activity within the cortex after removal of the stimulus.

The results of these experiments clearly demonstrate the existence of two distinct cortical mechanisms. The first of these exerts control over somatic movement as shown by the normal motor response to stimulation and the exaggerated activity of the epileptiform type of motor after-response. The second influences vasomotor and other visceral activities, and is demonstrated by the occurrence of heart rate and blood pressure changes during cortical stimulation in the absence of any somatic activity. Since the alterations in the vasomotor mechanism which would reasonably be expected to occur under cortical direction would be those concerned in adjusting the responses of the vascular system to the activities of the somatic, it is not surprising that the distribution of the representation of the vasomotor system so closely overlaps that of the somatic. In view of this close anatomical association it is quite possible that in the normally functioning individual afferent impulses which activate the somatic efferent will also affect the visceral efferent mechanism.

The abnormal visceral activity which precedes and accompanies an epileptic attack has been described by Frisch (22), and furthermore according to Watts and Frazier (23) such phenomena evidently occur in the absence of skeletal motor manifestations. Thus the cortical lesion responsible for the somatic manifestations of the clinical epileptic seizure may also initiate activity within the overlapping cortical visceral mechanism, and so account for the vegetative disturbances which precede and accompany the attack.

SUMMARY

1. These investigations were undertaken to verify by controlled cortical stimulation experiments the evidence for a cortical influence on the cardiovascular system.

2. Blood pressure and heart rate were recorded by mercurial or optical manometers during stimulation of the cerebral cortex of 40 cats, 18 monkeys and 1 chimpanzee under light ether anesthesia, and in many instances curarized to prevent muscular movements.

3. From the gyrus proreus and the sigmoid gyri in cats and the cortex adjacent to the superior precentral sulcus as well as scattered points in

the trunk and arm areas and on the superior, middle and inferior frontal gyri, and marginal and cingulate gyri anterior to the central sulcus in monkeys, rise of blood pressure, acceleration of heart rate, retraction of the nictitating membranes and dilatation of the pupils were recorded.

4. A decline of pressure frequently resulted from stimulation of the lateral, suprasylvian, ectosylvian and sylvian gyri in cats and occasionally the marginal gyrus and a small area of cortex near the anterior tip of the superior precentral sulcus in monkeys.

5. Marked slowing of the heart occurred in a few instances of cortical stimulation especially in animals from which the stellate ganglia and adrenal glands had been removed.

6. That these cardiovascular responses were initiated by stimulation of nervous elements situated within the cortex may be concluded on the basis of four lines of evidence: *a*, pressor and depressor points were discretely localized within 3 to 4 millimeters of each other; *b*, local anesthesia of the cortex to a depth of three millimeters abolished the responses; *c*, isolation of a small responsive area of the cortex by undercutting eliminated responses from this area; *d*, excitation of the efferent fiber tracts in the white matter immediately below the cortex normally gave a vasomotor response but none resulted from stimulation of these tracts when they had become degenerated as a result of previous removal of the overlying cortex.

7. These responses are not a part of an epileptiform muscular response since they can be elicited in non-curarized animals in which no muscular movement has occurred, and furthermore epileptiform type of muscular responses have been observed without change in blood pressure.

8. It is therefore concluded that there is a mechanism by which the cortex can influence the state of the cardiovascular system, and that through this mechanism the cortex may bring about a finer adjustment of the activity of the heart and circulation in accordance with the exigencies of the external environment and the immediate activities of the skeletal musculature. The existence of such a mechanism may explain the origin of the disturbances of the heart and circulation associated with the epileptic seizure.

The authors are grateful to Prof. John F. Fulton for his many suggestions and helpful criticism during this research.

REFERENCES

- (1) MINKOWSKI, M. *Rev. Neurol.* 59: 1177, 1933.
- (2) POPPER, L. *Deutsch. med. Wchnschr.* 59: 1163, 1933.
- (3) TAYLOR, N. B. AND H. G. CAMERON. *This Journal* 61: 385, 1922.
- (4) CHEVALLIER, P. E. *De la paralysie des nerfs vaso-moteurs dans l'hémiplégie.* Thèse, Paris, no. 175. Paris, Martinet, 1867.

- (5) FULTON, J. F. *Proc. Inst. Med. Chicago* 2: 1, 1936.
- (6) PINKSTON, J. O., P. BARD AND D. McK. RIOCH. *This Journal* 109: 515, 1934.
- (7) KENNARD, M. A. *Arch. Neurol. Psychiat.* 33: 537, 1935.
- (8) SCHIFF, M. *Arch. f. exper. Path. u. Pharmacol.* 3: 171, 1875.
- (9) DANILEWSKY, B., JR. *Pflüger's Arch.* 11: 128, 1875.
- (10) BARD, P. *Arch. Neurol. Psychiat.* 22: 230, 1929.
- (11) LANGWORTHY, O. R. *Contributions to Embryology*, Carnegie Inst. Washington 19: 177, 1927.
- (12) LANGWORTHY, O. R. *Bull. Johns Hopkins Hosp.* 42: 20, 1928.
- (13) CAMPBELL, A. W. *Histological studies on the localisation of cerebral function*. Cambridge, University Press, 1905.
- (14) FRANÇOIS-FRANCK, C.-E. *Leçons sur les fonctions motrices du cerveau* Paris, Octave Doin. 1887.
- (15) METTLER, C. C., W. F. HAMILTON, L. ALLEN, R. A. WOODBURY AND F. A. METTLER. *Anat. Rec.* 64 (Suppl. 4): 33, 1936.
- (16) WEBER, E. *Der Einfluss psychischer Vorgänge auf den Körper, insbesondere auf die Blutverteilung*. Berlin, Julius Springer, viii, 426 pp., 1910.
- (17) BECHTEREW, W. VON. *Die Funktionen der Nervencentra*. Jena, Gustav Fischer, 3 Bd., 1911.
- (18) DUSSER DE BARENNE, J. G. AND F. KLEINKNECHT. *Ztschr. f. Biol.* 82: 13, 1924.
- (19) HUNSICKER, W. C. AND E. A. SPIEGEL. *Proc. Soc. Exper. Biol. Med.* 31: 974, 1934.
- (20) SCHÄFER, E. A. *Text-book of physiology*. Edinburgh, Pentland, 2 vols., 1900.
- (21) CROUCH, R. L. AND K. THOMPSON. *Anat. Rec.* 64: (Suppl. 4) 11, 1936.
- (22) FRISCH, F. *Das "vegetative System" der Epileptiker*. Berlin, Julius Springer, 1928.
- (23) WATTS, J. W. AND C. H. FRAZIER. *J. Nerv. Ment. Dis.* 81: 168, 1935.

STRUCTURAL AND FUNCTIONAL ORGANIZATION OF THE CENTRAL MECHANISM CONTROLLING BREATHING^{1,2,3}

ROBERT GESELL, JOHN BRICKER AND CONWAY MAGEE

From the Department of Physiology, University of Michigan, Ann Arbor

Received for publication June 11, 1936

Probably the first recorded opinion on the localization of the control of breathing is that of Galen who reported instantaneous death from section of the cord at the level of the first and second vertebrae. The same effects were again noted by Lorry in 1760 on dividing the cord between the second and third vertebrae. Legallois (1812) then designed transection experiments for more precise localization of respiratory control and concluded that breathing is dependent upon a particular and small part of the medulla near the origin of the pneumogastric nerve. These experiments mark the beginning of an augmenting accumulation of valuable, but often conflicting, data on a most involved subject.

The need of more exact information on the localization of the central controlling mechanism of breathing and on the structural organization and function of its component cell groups scarcely requires comment. To fully understand the train of events leading to a coordinated respiratory act, it is quite essential to follow the process from the sensory receptors, through the central nervous system, and to the motor end organs, for until we know the units participating we cannot study effectively the processes involved. The electrical method which tells where and how a cell is acting seems to offer a direct and supplementary attack.

METHOD. Four phases of this problem have required our special attention: the finding of respiratory potentials; the registration and study under various respiratory conditions; the histological localization of the source of potentials; and the interpretation of results.

Finding respiratory potentials. Our experiments were done on dogs anesthetized with morphine and urethane or with evipal.⁴ The skull cap was resected and in most of the experiments the cerebral hemispheres and

¹ Preliminary report—Proc. Soc. Exper. Biol. and Med. **32**: 787, 1935.

² Preliminary report—Proc., This Journal **113**: 48, 1935.

³ This study was supported in part by grants from the National Research Council and the Rockefeller Foundation.

⁴ This anesthetic was supplied by Winthrop Chemical Co., New York, N. Y.

portions of the cerebellar vermis were removed by suction. In the experiments, confined mainly to the study of potentials in the mesencephalon and diencephalon, where evipal was used, we may assume little or no chemical anesthesia throughout our observations, but in the experiments in which morphine and urethane were employed we must assume chemical action of the anesthetics after, as well as before, decortication.

The exposed brain stem was explored for respiratory potentials with two fine insulated needle electrodes, bare at the tips, separated 1.5 to 2 mm. along the longitudinal axis of the animal. The electrodes were mounted on a specially constructed and calibrated manipulator, allowing movement in the longitudinal and transverse directions and penetration in the dorso-ventral direction. The electrodes were connected, through amplifiers, with a loud speaker for auditory detection and with a General Electric Multiple Oscillograph for visual inspection and registration of potentials. The dogs were placed in the prone position with the head firmly fixed in a specially devised head holder to avoid artefact potentials of mechanical origin. Electrical interferences were avoided when necessary by shielding with an adjustable aluminum shield.

With graded penetration there was an ever changing medley of action sounds—some weak, others loud; some discrete, others indiscrete, i.e., out of phase with one another; some of regular frequency, others of irregular sequence; some of high frequency and some of low frequency; and some fluctuating with the respiratory rhythm.

We may assume with a fair degree of certainty that potentials showing fluctuations in rhythm with respiration are in some way related to respiration. On the other hand, we cannot conclude with equal certainty that potentials failing to display such fluctuations are unrelated to respiration. This constitutes, for the time, a more uncertain angle of the problem.

In our earlier experiments we studied only potentials showing respiratory rhythm. In searching for these potentials we adopted the procedure of inserting the electrodes at 1 mm. intervals along parallel and transversely directed lines 2 mm. apart. With each penetration from the dorsal to the ventral surface, the electrodes were inserted at steps of 0.1 to 0.2 mm. and after each advancement of the electrodes an interval of several respirations was allowed for the auditory detection of respiratory potentials. A complete exploration of the medulla and upper cord was slow and tedious, yet a thorough exploration seemed quite essential. First of all, the electrodes are highly selective and tap potentials only in their immediate vicinity, thus unless cells are discharging en masse they could easily be missed; secondly some of the structures supposedly participating in the respiratory act are very small; and thirdly respiratory potentials at best were relatively hard to find.

When respiratory potentials were encountered they were photographed

along with changes in tracheal pressure and tidal air. Time was recorded in 0.04, 0.20 and 1.0 second intervals and in many experiments mean blood pressure and the pulse were registered as well (see figs. 2-7). The procedure is similar to that previously described in studies on the action potentials of respiratory muscles (Gesell, 1936a). Downstroke represents inspiration in both tracheal pressure and tidal air records and upstroke represents expiration. The tracheal pressure record gives the change in phase of breathing with accuracy but the tidal air tracing shows considerable lag.

After having completed any set of observations at a single setting of the electrodes, a lesion was placed by passing a momentary weak galvanic current across the electrodes at the site of the potentials. Each lesion was entered in the protocol with respect to gross position determined by the three dimensional manipulator to insure later positive histological localization. At the close of each experiment the dog was killed, the brain stem was carefully removed and fixed in trichloroacetic acid, mercuric chloride and alcohol, embedded in paraffin, and cut into 20 micra sections. Every fifth section was mounted in serial order and stained in toluidin blue according to the method described by Huber (1927).

The lesions were approximately 0.5 mm. in diameter. Occasionally they were difficult to find due to small hemorrhages from the rupture of minute blood vessels by the needle electrodes. In such doubtful cases of identification experimental findings were disregarded. The highly expert judgment of Doctor Crosby on the possible nervous structures involved in the lesions gives us confidence in the anatomical phase of our problem.

Gross localization of respiratory potentials. We have studied the earlier experiments of others and have presented condensed discussions, under appropriate headings, of those results which seem essential to the interpretation of our findings. These discussions may be followed to better advantage by reference to our sketch of the medullary nuclei (fig. 10) as constructed from serial sections of the brain stem of the dog. A more extensive review, including the higher "respiratory centers" is available in the excellent discussion *Le Centre Respiratoire* by Cordier and Heymans (1935). See also the recent review by Finley (1931).

Since the transections of the brain stem by Legallois abundant evidence has accumulated indicating that the primary control of breathing resides in the medulla. Charles Bell (1833), mainly from clinical observations, concluded that "In the lateral portion of the medulla oblongata, from which the several respiratory nerves go off, there is seated a power which passing through the diverging nerves combines the remote organs." Flourens (1842-51), with experimental methods, limited the center to his "noeud vital" near the obex of the medulla. Longet (1847), Mislowsky (1885), Laborde (1890), Gad and Marinescu (1892), Arnheim (1894), Kohnstamm (1900), Bechterew (1908), Finley (1931), Johnston (1932)

all placed the seat of control in the medulla. Their experiments will be reconsidered in relation to the formatio reticularis. Schiff (1858), Gierke (1873) and Girard (1890) reached similar conclusions. Their findings will be reconsidered in connection with the alae cinereae.

Kronecker and Marckwald (1880) and Marckwald (1887) transected the medulla at the cephalic end without noticeable effects on breathing. At a decapitation (between the 4th and 5th vertebrae) Gad in 1886 (quoted from Kronecker, 1887) saw the trunk fall limply while the head made dyspneic movements for $1\frac{1}{2}$ minutes. Paralysis of breathing by cooling (Fredericq (1883), Trendelenburg (1910), and Nicholson (1936a)) and by local application of cocaine hydrochloride to the floor of the fourth ventricle (Aducco (1890), Franck (1892), Langlois and Garrelon (1908), and Nicholson (1936b)) indicated that the primary respiratory center resides in the medulla and dominates the cord.

Grossmann (1889) proposed a reciprocal association of several respiratory centers as opposed to a complete subordination of various centers to a primary medullary center. According to Trevan (1916) the respiratory center is a chain of neurones extending along the entire floor of the fourth ventricle and removal of any part affects coördinated breathing. He suggested with Boock that while the medullary center is primitive, complete dependence of breathing on the vital node must be sought in some animal lower than the frog. Springer (1928) believes that the selachians possess a central motor mechanism of respiration located in the medulla. Hyde (1894) concludes that each ganglion of the cord of the *Limulus* constitutes an automatic and reflex center for the respiratory movements; that lesion or loss of part of the entire brain, esophageal collar, or post-esophageal ganglion has no influence on the respiratory movements; that after severing the cord the anterior and posterior divisions show the same respiratory response to exercise, feeding, and irritation. She finds a higher development of the respiratory center in the skate (1904), intermediate between the simple segmental arrangement of invertebrates and the complex modified and specialized centers existing in higher vertebrates. According to her the respiratory center of the skate occupies definite sensory and motor areas in the medulla; the movements are segmental but the relationship of the respiratory organs and their segmental centers is not so obvious as in the lower forms; the developmental changes of shifting and consolidation have begun to mask the segmental connection of the brain; and where development has proceeded a step further this connection would be demonstrated only with difficulty.

The recent findings and conclusions of Lumsden (1923) are particularly interesting in relation to this concept of increasing complexity of the respiratory mechanism with evolutionary development. Modifications of breathing produced by transection of the brain stem of the cat led Lumsden

to postulate four highly specialized centers. A gasping center below the *striae acusticae* provides the primitive respiratory act composed of a deep, short, active inspiration followed immediately by a passive expiration. This act is in turn modified by higher centers. The most caudal of these centers is the expiratory center which adds active expiration. Next comes the apneustic center which prolongs the period of inspiration producing apneustic breathing and finally the pneumotaxic center which periodically interrupts apneusis and produces normal rapid breathing.

Henderson and Sweet (1929) attributed Lumsden's results to involvement of the rubro-spinal tract. Keller (1931) disagreed with Henderson and Sweet. Nicholson (1936a) states "The fact that a marked apneusis may be brought about by a moderate cooling of a small area in the region of the *calamus scriptorius* seems at least to remove the necessity for the existence of a pneumotaxic center in the upper pons with the specific function of interrupting inspiration. It appears that inspiration is normally rhythmically interrupted by the activity of some mechanism located just anterior to the apex of the *calamus scriptorius*." To these various criticisms may be added that of Barcroft (1934) who has accepted the gasping center as an established fact but explains apneustic breathing by a reduction of normal proprioceptive impulses which automatically function to cut each inspiration short. In more recent experiments, Henderson and Craigie (1936) again fail to confirm the findings of Lumsden and conclude from a series of varied lesions that the respiratory area appears to lie in the medial third of the medulla, limited above approximately at the junction of the middle and upper thirds of the hypoglossal nucleus and below by the pyramidal crossing.

We have done some eighty experiments dealing with the localization of the central respiratory mechanism by means of electrical sounding. In sixty of these, the explorations were confined to the medulla and upper cervical region of the cord between transverse sections 12 mm. above and several centimeters below the obex. In the remaining twenty, the explorations were limited to the thalamus, hypothalamus, mid-brain, pons and medulla above a level 8 mm. cephalad to the obex. A few explorations were made in the cerebellum but none were made above the thalamus.

The results of the first group of experiments are presented in figures 1A and B in which the location of both inspiratory and expiratory potentials are plotted to scale. Inspiratory potentials are designated by an *O* and expiratory by an *X*; where both occurred along one penetration of the electrodes they are designated by an *X* enclosed in an *O*. The medial and lateral reticular nuclei are also plotted in figure 1A as accurately as their diffuse structures will permit and the accompanying figure 1B shows the important respiratory nuclei plotted to the same scale. Neither the depth of potentials nor the depth of the nuclei is indicated. The histological localiza-

TABLE 1

Tabulation of structures involved in lesions associated with respiratory and continuous potentials

	INSPIRATORY	EXPIRATORY	CONTINUOUS
Brachium conjunctivum	49/4 (I-5)		
Descending root of V	14/8 (P-8)		
Nucleus of	51/2 (J-2)		
Dorsal funiculus of spinal cord	57/2 (G-3)	19/4 (C-2)	
		22/2 (A-3)	
Dorsal root fibers	19/1 (B-1)	19/1 (B-1)	23/2
		57/3 (H-5)	
Collaterals of	15/2 (C-1)	15/2 (C-1)	
	21/3 (A-2)	37/1 (H-4)	
Commissural fibers from	49/1 (I-3)		
Dorsal root fibers and edge of dorsal funiculus		22/3 (A-4)	
Fasciculus solitarius			28/4
			56/4
Nucleus of	27/6 (N-4)		
	12/1 (P-5)		
Fibers from nucleus to dorsal efferent	36/4 (O-4)		
Nucleus of X			
Internal arcuate fibers	27/1 (S-1)		
(These lesions fall on prominent bundles of arcuate fibers)	46/1 (O-5)		
	47/3 (M-2)		
	48/3 (L-2)		
	50/1 (M-4)		
	50/3 (N-5)		
	51/1 (J-3)		
	55/1 (O-6)		
	57/5 (M-6)		
Lateral funiculus of spinal cord	56/2 (I-1)		
Lateral reticular nucleus	50/4 (N-6)		
Lateral reticular nucleus and internal arcuate fibers	47/5 (O-7)	18/4 (T-2)	
Lateral reticular nucleus and lateral reticulo-spinal tract	56/5 (M-7)		
Lateral reticulo-spinal tract	14/1 (D-1)	22/1 (A-1)	
	19/2 (B-2)	49/3 (J-5)	
	19/5 (C-3)		
	19/6 (D-4)		
Lateral reticulo-spinal tract and internal arcuate fibers	44/5 (H-8)		
Lateral reticulo-spinal tract and dorsal horn	21/2 (A-5)		
Lateral reticulo-spinal tract and ventral horn cells	57/1 (E-1)	36/2 (H-7)	
Medial lemniscus	47/6 (O-2)	31/3 (N-1)	
Medial lemniscus and olivo-cerebellar fibers	36/1 (Q-2)		

TABLE 1—Continued

	INSPIRATORY	EXPIRATORY	CONTINU- OUS
Medial reticular formation	31/4 (Q-4)	35/2 (P-6)	26/1 27/4 28/2 28/3 35/6
Medial reticular formation and internal arcuate fibers	14/4 (J-4) 52/1 (Q-3) 52/2 (Q-6) 54/2 (T-1)	14/4 (J-4) 25/2 (K-2) 35/1 (Q-5)	23/6 27/7 32/1
Medial reticular formation and medial lemniscus			38/1
Medial reticular formation and root fibers of XII			27/5
Medial reticulo-spinal tract	24/1 (N-7)		
Medial reticulo-spinal tract and medial tecto-spinal tract			48/2
Medial tecto-spinal tract	31/1 (P-2) 31/1 (N-2)		
Medial tecto-spinal tract and medial lem- niscus			27/2
Mesencephalic root of V	51/3		
Nucleus ambiguus	47/4 (M-1) 57/6 (O-8)	35/5 (K-1)	
Nucleus ambiguus and lateral reticulo- spinal tract	12/2 (P-7) 28/5 (K-3) 47/1 (L-3) 47/2 (L-3)	14/5 (J-1) 28/5 (K-3)	
Nucleus cuneatus	44/2 (M-5)		23/4
Nucleus gracilis	23/1 (H-3)		
Nucleus of VI			26/2
Nucleus of XI (dorsal efferent)			25/1
Nucleus of XII	24/1 (N-3) 56/4 (M-3)	27/3	32/4
or fibers from fasciculus solitarius to Nucleus of XII	42/1 (L-1) 24/2 (O-4)	24/2 (O-4)	
root fibers from		25/3 (P-3)	48/1
Olivary nucleus			29/4
Red nucleus	50/5		
Rubro-spinal tract and cerebello-reticu- lar fibers	49/5		
Ventral horn cells	14/2 (D-3) 22/3 (A-4) 44/4 (I-7) 48/5 (G-1) 56/1 (H-1)	14/3 (H-2) 48/4 (G-2) 49/2 (I-5) 56/6 (I-6)	

TABLE 1—*Concluded*

	INSPIRATORY	EXPIRATORY	CONTINU- OUS
Ventral horn cells and commissuro-cerebellar fibers		18/3 (D-2)	
Ventral horn cells and proprius bundle	15/5 (I-2)		
Ventral reticulo-spinal tract			29/1
Ventral reticulo-spinal tract and fibers of cervical nerve I		44/1 (H-6)	
Ventral reticulo-spinal tract and olivary nucleus	57/4 (O-1)		29/3
Vestibular nucleus			29/2

tion of respiratory potentials, therefore, cannot be determined with the aid of these figures and will be considered later. The figures are of interest in showing the proximity of respiratory potentials to well known nuclear structures in the medulla. Respiratory potentials will be seen to have been encountered most frequently in the region of the obex. After passing a level approximately 5 mm. above the obex they were less frequent and barring occasional exceptions they were absent 10 mm. above the obex, a level corresponding to the cephalic limit of the inferior olivary nucleus and the exiting root fibers of the vagus nerve. This level may temporarily be accepted as the upper limit of the main respiratory mechanism in the medulla.

When we attempt to limit the lower border of this mechanism we meet with a different situation. As might well be suspected, respiratory potentials are found at any level of the cord where respiratory muscles are represented. Our schema, therefore, fails to show a lower envelope of respiratory potentials limiting the lower border of the medullary respiratory mechanism. With the use of table 1, however, we are able to identify the histological source of the potentials and in listing these sources from above downward we reach a level approximately 2 mm. below the obex (in dogs weighing 14 kgm.) where potentials arising in the reticular cells cease. Anatomically this level corresponds to the cephalic limit of the pyramidal decussation, the caudal pole of the lateral reticular nucleus of the medulla oblongata and a point slightly cephalad to the exiting root fibers of the first cervical nerve. This may possibly mark the lower limit of the so-called respiratory center of the medulla.

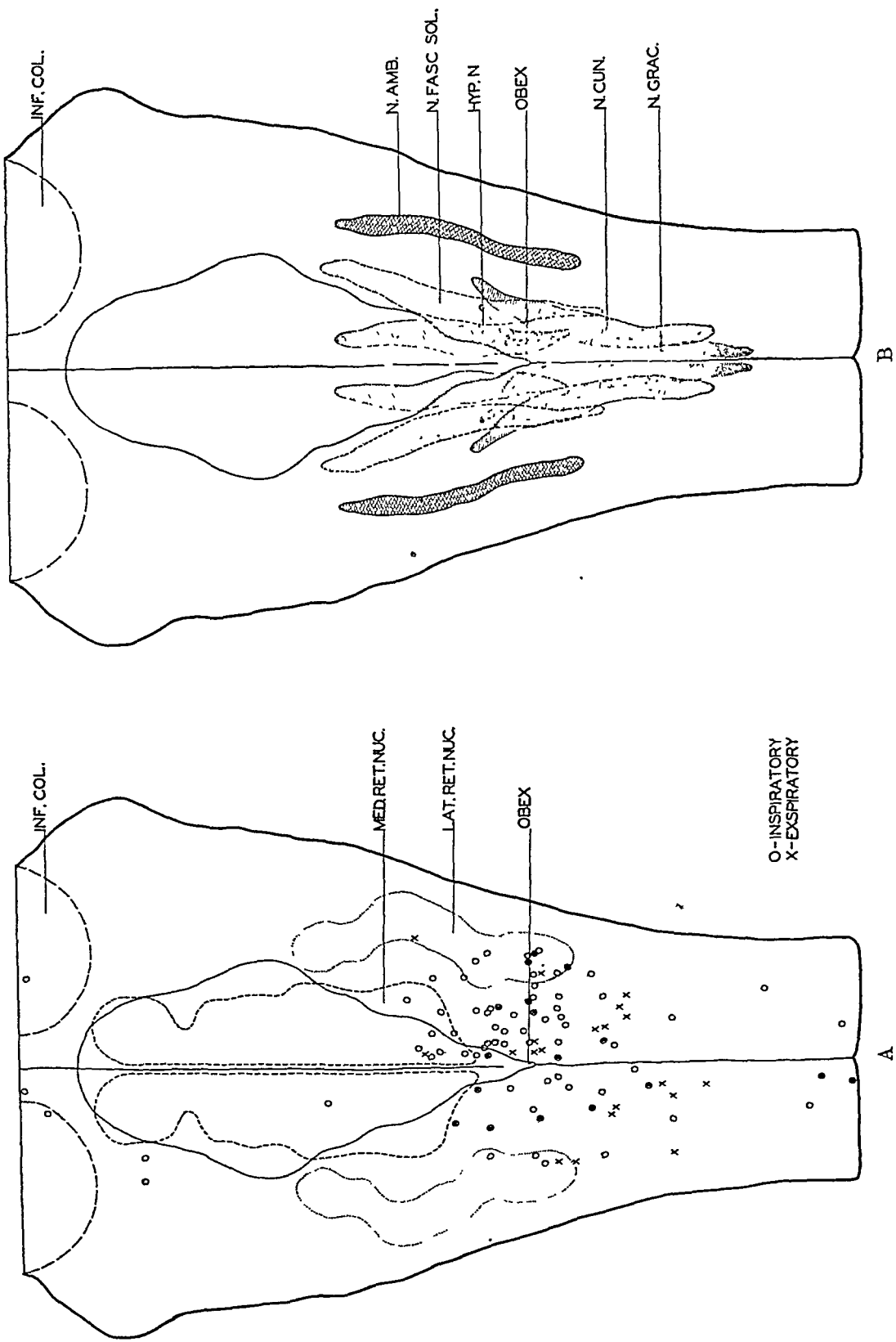
Higher respiratory centers. Many authors have removed the influence of the mid-brain without seriously affecting the mode of breathing. Among these are Legallois (1812), Flourens (1842-51), Longet (1868), Kronecker and Marckwald (1880), Marckwald (1887), Mislowsky (1885), Asher and Luscher (1899), Bazett and Penfield (1922), Lumsden (1923), Spiegel and Enghoff (1925), Schoen (1928), Teregulow (1929), Henderson and Sweet

(1929), Henderson and Craigie (1936). On the other hand a large group have found that removal of all or part of the mid-brain permanently changes respiration. In this group are Martin (1878), Lewandowsky (1881 and 1896), Asher and Luscher (1899), Joukowski (1899), Nicolaides (1905), Trevan (1916), Coombs and Pike (1918), MacLeod (1919), Trevan and Boock (1922), Keller (1928-9), and Coombs (1930). However, if the section is just below the corpora quadrigemina and the vagi are also cut then respiration is invariably affected according to Marckwald (1887), Loewy (1888), Macleod and Page (1922), Lumsden (1923), Trevan and Boock (1922) and Henderson and Sweet (1929). [Nicholson obtains the same effect by cooling in the region of the obex after vagotomy (1936a).] Nonne (1923), Stern (1923), Pette (1923), Lotmar (1926), and Hess and Pollak (1927) from studies of various diseases in which breathing is affected believe that the mid-brain has some regulating control of respiration. Ferrier (1876), Martin (1878), Martin and Booker (1878-79), Christiani (1880), Knoll (1885), Joukowski (1899), Brown (1914) and Coombs (1920) obtained varied effects on breathing upon stimulation at different locations in the mesencephalon.

Transection immediately below the thalamus has no effect upon breathing according to Legallois (1812), Flourens (1842), Longet (1868), Mislawsky (1885), Marckwald (1887), Loewy (1888), Asher and Luscher (1899), Coombs (1918), Trevan and Boock (1922), Schoen (1928), Keller (1929) and Henderson and Sweet (1929). Changes in breathing produced by stimulation of the thalamus have been frequently described (Christiani, 1885; Knoll, 1885; Arnheim, 1894; Joukowski, 1899; Bechterew, 1899; Sachs, 1911; Smith, 1926; Schoen, 1928; and Ransom and Magoun, 1933).

Keller and Hare (1932) produced polypnea by removal of the hypothalamus. Karplus and Kreidel (1909-1910), Asher (1916), Keller and Hare (1932), Ransom and Magoun (1933), Leiter and Grinkler (1934) and Ransom, Kabat, and Magoun (1935), Ectors and Brookens (1936) describe respiratory effects upon stimulation of the hypothalamus.

Considering the accessibility of the respiratory mechanism to almost every variety of impulse—the higher reflex connections of the lower spinal and medullary reflex arcs, the effects of pain, heat, cold, and proprioceptive impulses, and the voluntary and emotional control of breathing—it is only reasonable to expect effects upon breathing from interruption and stimulation of various reflex and fiber tracts. It seemed to us that if there are important mechanisms above the medulla which under all conditions determine the rate and depth of breathing we might be fortunate enough to find these locations and study their function. The region to be explored is considerably greater than that about the obex and, since a complete exploration such as that described for the lower medulla was precluded by the inevitable deterioration of the animal, a staggered exploration was em-



Figs. 1A and 1B. Gross localization of respiratory potentials in relation to nuclear structures.

ployed. Explorations were started at the upper end of the thalamus and soundings were made at 1, 3, 5, 7, 9 and 11 mm. lateral to the mid-line and 2 mm. caudad, at 2, 4, 6, 8 and 10 mm. lateral to the mid-line. After moving the electrodes 2 mm. caudad again, a third series of explorations similar to the first was conducted. In the next animal the initial series of explorations was started, to the best of our measurements, 1 mm. lower or higher than the first explorations of the preceding experiment. In some animals death prevented a complete set of observations; in others, complete soundings were made on one side of the brain. Most of the observations were on the left side.

As in the explorations of the medulla, there was an abundance of miscellaneous potentials but only in five instances did any of them show a respiratory rhythm. These locations are roughly shown in figure 1A. Whether we have employed the proper procedure for locating respiratory potentials in these higher levels remains for future experiments. As was pointed out above an evanescent anesthesia (evipal) was used for these experiments as a precaution against excessive depression at higher levels.

Obviously the findings of those who described no effects on breathing upon transection at the cephalic end of the medulla speak strongly for residence of the principal or pace-setting center in the medulla and at no higher level. The effects of stimulation in the thalamus, hypothalamus and mesencephalon need not necessarily be interpreted in terms of higher "centers." They could be interpreted as indicative of regulative mechanisms which influence the activity of the medullary mechanism. According to Sachs (1911) "There is no center controlling respiration in the optic thalamus.—Any changes in respiration observed on stimulation are reflex effects."

Anatomically there are several theoretical circuits of respiratory impulses which we hoped to encounter (Huber and Crosby, 1929). The scarcity of respiratory potentials may allow several conclusions: During a steady state of anesthesia the upper levels may exert very little regulative action on breathing; some step in our procedure may have led to a reduction or disappearance of higher respiratory potentials; the paths of the impulses may be sufficiently restricted to evade our method of exploration. Despite the fact that twenty experiments were performed we are forced to admit the third possibility through our experience with medullary potentials since in a few of these experiments the most careful exploration failed to reveal respiratory potentials.

Histological localization of respiratory potentials. We have listed potentials in table 1 which have been positively identified with the galvanic lesions placed at the site of these potentials and in figures 8A and B and 9A and B the respiratory potentials are histologically localized with miniature triangles. The potentials are tabulated alphabetically with respect to

possible structures involved and classified in three columns as inspiratory, expiratory, and continuous without respiratory rhythm. The parenthetical notations identify each set of potentials histologically in the cross sectional drawings of figures 8 and 9.

In attempting our electrical study of the respiratory mechanism we were at first satisfied with a gross localization of respiratory potentials which might limit the boundary of the respiratory mechanism, but as the problem grew and the technique improved we were eager to refine the localization of potentials to microscopic or cellular units. It was quite apparent from our observations that the electrodes were very selective. The slightest advance in the penetration produced a readily observable change in the potentials. An up or down movement of 0.1 to 0.2 mm. might call forth an alternate appearance and disappearance of respiratory potentials. However, the limitations of our histological localization are set by the size of lesions we have found advisable to place. Lesions of approximately 0.5 mm. in diameter in many localities are likely to involve more than one group of structures, for example, lesions through a heavy bundle of internal arcuate fibers might possibly involve other structures in the reticular formation. A lesion at the edge of a nucleus might involve a few, but not negligible, units of other nervous elements. The question then arises as to the source of the potentials. Where both cellular and fiber structures are concerned, off-hand preference might be given to cellular source but we would rather hold a guarded position on this point. Though fiber tract potentials are frequently of a weaker order, they may at times record big deflections when neighboring cellular potentials are positively excluded, see for example the centrally placed lesion in the descending root of V (fig. 9A—P8). The conservative identification presented in table 1 seems to be the safer procedure.

The first point to attract attention is the diversity of structures involved in the chain of respiratory impulses. For convenience these potentials may be temporarily grouped under four headings—those obtained in the sensory structures in the medulla and cord, those in the intermediary reticular formations, those in the connecting tracts between the reticular formations and the motor cells of the respiratory muscles, and finally those in the motor cells at the end of the chain. Figures 2A and B and 3A and B show impulses on the sensory side, figures 4A and B and 5 are concerned with the intermediary reticular stations, figures 6A and B with the region of the lateral and medial reticulo-spinal tracts and figures 7A and B with motor stations in the nucleus ambiguus and the ventral horn of the cord. These figures serve only as examples of outstanding phases of the work. The major results have been presented in condensed form in table 1 with very little discussion. Their more complete analysis is left to the reader.

Figure 2A represents a group of incoming sensory signals registered in

the nucleus cuneatus during normal breathing, (for localization of potentials see fig. 9A—lesion M5). They are distinctly discrete, rising in rate during inspiration to 35 per second, and falling abruptly to zero when expiration supervenes. The signals in figure 2B were registered from the nucleus and fasciculus gracilis (fig. 8B—H3). They are from another dog and differ from those of figure 2A in their continuity and lower rate and in

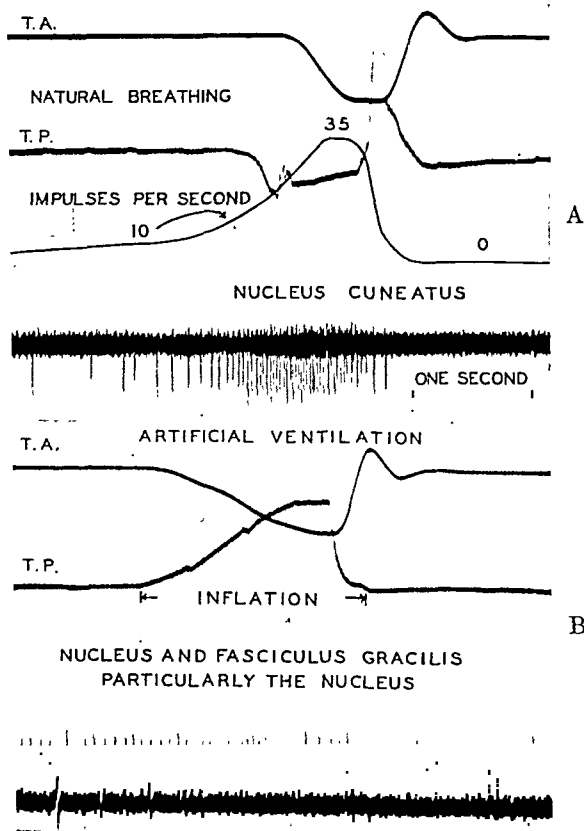


Fig. 2A. Inspiratory signals recorded from the cuneate nucleus during normal breathing.

Fig. 2B. Inspiratory signals recorded from the gracile nucleus during artificial ventilation.

the fact that they occur during artificial respiration administered after complete failure of breathing. The signals are similar to those in figure 2A in that they appear and accelerate during filling of the lungs, suggesting that in both instances they are of proprioceptive origin.

The nucleus fasciculus solitarius acting as the sensory receiving station for vagal impulses might be expected to show a discharge during inspiration when the proprioceptive endings of the lungs are stimulated. Figure 3A

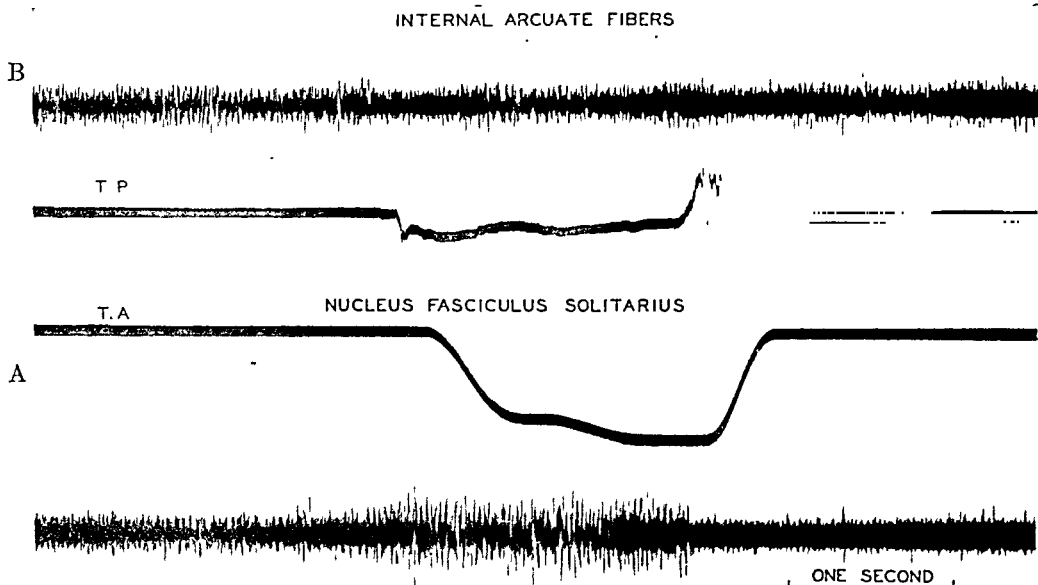


Fig. 3A. Inspiratory signals recorded from the nucleus fasciculus solitarius
 Fig. 3B. Inspiratory signals recorded from internal arcuate fibers

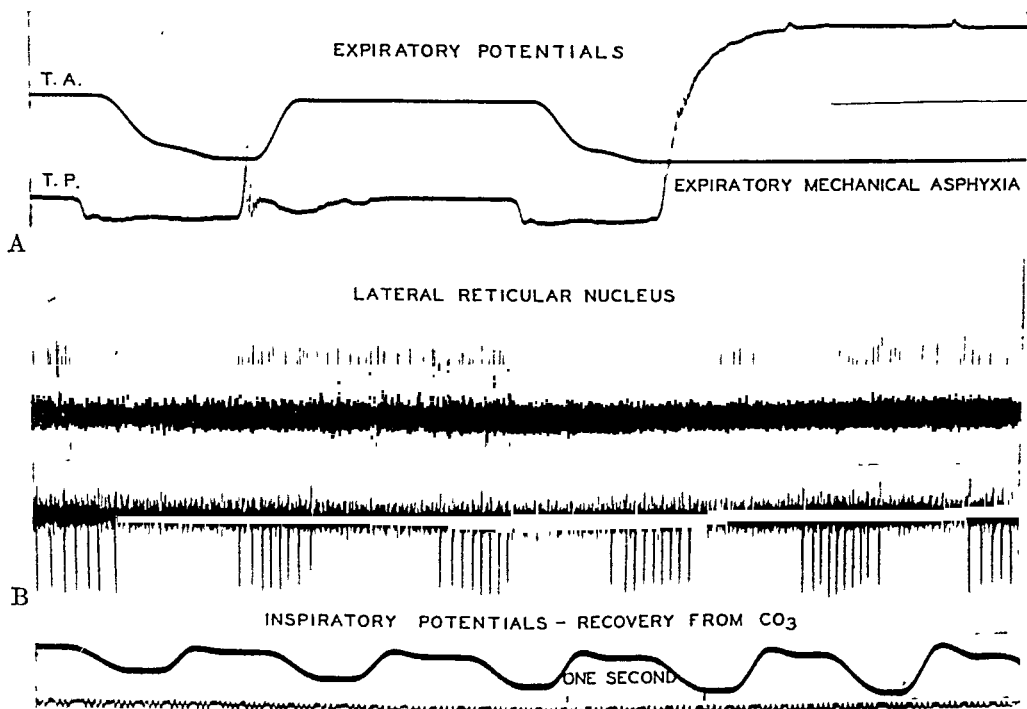


Fig. 4A. Expiratory potentials of the lateral reticular nucleus during normal breathing and expiratory mechanical asphyxia.

Fig. 4B. Inspiratory potentials of the lateral reticular nucleus showing recovery from intravenous injection of sodium carbonate.

shows a volley of signals increasing at the beginning of inspiration and thus agreeing with the linear relation of rate of discharge to lung inflation established by Adrian (1933). But whether the discharge of the nucleus fasciculus solitarius continues to conform with theoretically augmenting incoming signals during further lung extension is only suggested in the heavy discharge. This point is worthy of further study but up to the present we have encountered potentials of the nucleus and fasciculus solitarius only on three occasions despite our large number of medullary soundings.

Another group of sensory signals is indicated in figure 3B where a lesion was placed at the site of inspiratory potentials in the path of a heavy bundle of arcuate fibers (fig. 9A—N5). These potentials conceivably are of proprioceptive origin on their way to higher centers after relay in the nuclei cuneatus and gracilis. They are very indiscrete and in that respect differ from the regular, well defined potentials so commonly found in the reticular gray substance. The recent description of Mingazzini (1928), indicating collateral connections between the cuneate and gracile nuclei and the reticular cells, emphasizes an important stream of signals for the control of breathing.

The reticular formations are undoubtedly in the pathway of respiratory signals. When electrodes are properly placed, that is, in the vicinity of an active unit, the potentials are usually discrete, of large magnitude, and easily recorded. They occur during expiration, as in figure 4A (fig. 8A—T2), and during inspiration, as in figure 4B (fig. 9A—N6). They vary considerably in rate and regularity of discharge. In figure 4b the signals are slower and more regular than in figure 4A. In some instances they are as slow as 30 per minute. They are modified by conditions affecting the respiratory act. If a dog is allowed to inspire, but denied expiration, a reflex slowing of breathing occurs which is accompanied by a prolonged activity of expiratory muscles. Since muscle action potentials persist throughout the expiratory pause, the prolonged firing of the lateral reticular nucleus in figure 4A is worthy of passing comment. So, too, are the effects of intravenous injection of sodium carbonate on the firing of the lateral reticular nucleus in figure 4B which shows recovery from an injection of sodium carbonate. Inspiration at its smallest volume shows 7 slowly repeating discharges per inspiration at the rate of 15 per second and as the tidal air increases the impulses step up to 10 per inspiration and the rate accelerates up to 20 per second. Within nine breaths beyond the limits of the figure the discharge is back to fifteen per inspiration and a rate of 27 per second. The results compare in many respects with those obtained on inspiratory muscles.

A good example of continuous but irregular potentials from a slowly discharging reticular formation (in this instance medial) is illustrated in

figure 5A. The potentials might easily be classified as inspiratory but for the fact that these evanescent accelerations occur in preceding breaths without regard to phase of breathing. Yet, as figure 5B, obtained from the succeeding breath, shows, these potentials are susceptible to modification into an orderly, discontinuous, expiratory rhythm. Inspiratory mechanical asphyxia practically stopped the discharge during the phase of inspira-

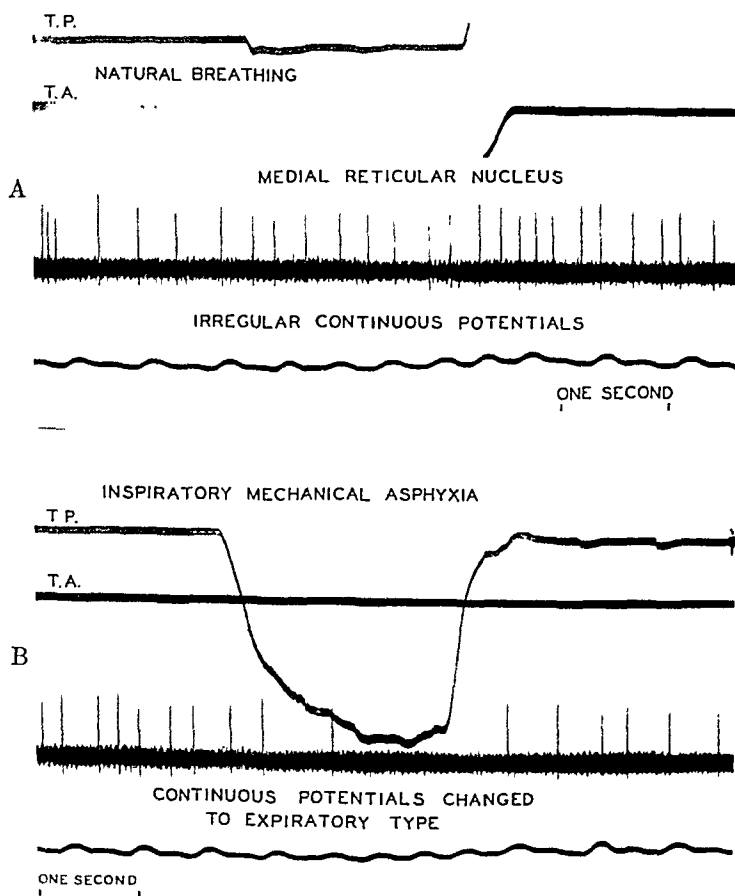
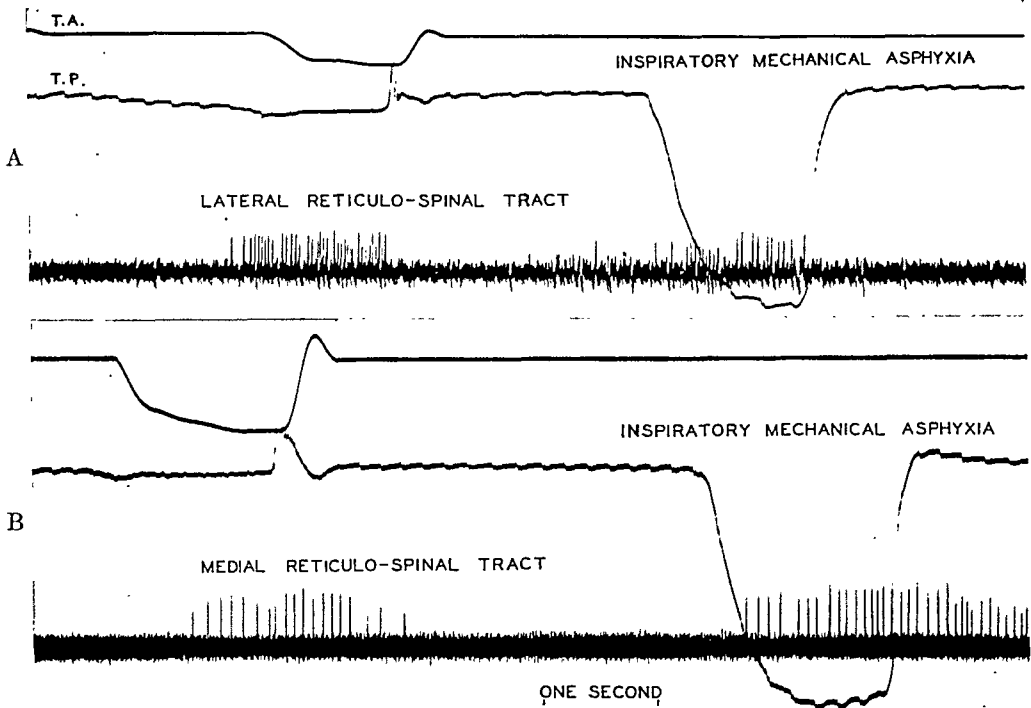


Fig. 5. Continuous potentials of irregular sequence in the medial reticular nucleus changed to the expiratory type by inspiratory mechanical asphyxia.

tion. This state of firing persisted for 7 respiratory cycles and within three breaths after allowing inspiration the potentials were back to their irregular, continuous sequence.

Papez (1926) has described three reticulo-spinal tracts—lateral, medial, and ventral—joining the reticular formations of the medulla, pons and mesencephalon with the cord. These are represented diagrammatically in figure 10. Respiratory signals have been tapped from all of these tracts,

both in the medulla and cord. Inspiratory signals from the medial reticulo-spinal tract are reproduced in figure 6B (9A—N7) and from the lateral reticulo-spinal tract in figure 6A (fig. 9B—3C). The potentials are not always as discrete as shown in these figures but generally resemble those of the reticular formation proper. In figure 6B the inspiratory discharge persists for a long period into the expiratory phase. During inspiratory mechanical asphyxia the potentials are augmented in rate and persist still longer into the expiratory phase. In figure 7B the acceleration during inspiration is less and the augmentation during inspiratory mechani-



Figs. 6A and B. Inspiratory potentials of medial and lateral reticulo-spinal tracts showing effects of inspiratory mechanical asphyxia.

cal asphyxia seems to be missing. This is an unusual response if compared with the responses for an inspiratory muscle (Gesell, 1933).

Respiratory impulses on the efferent side of the arc are shown in figures 7A (fig. 9A—M1) and B (fig. 8B—I2). Potentials from the nucleus ambiguus and from the anterior horn cells are both inspiratory and characteristic for contraction of voluntary muscle (Adrian and Bronk, 1928) and conform with the triangular fusillades of inspiratory muscles described by Gesell (1936B). In the upper record, two motor cells from the nucleus ambiguus are contributing potentials while in the lower record only one ventral horn cell is recording. In the latter record this ventral horn cell

is decreasing in activity and shortly retires completely. Later, mechanical asphyxia recalls it along with neighboring cells into the firing line.

Alae cinereae. Workers in the control of breathing have directed their experiments not only to regional localization but to structural localization as well. Many of their conclusions, as indicated before, have been

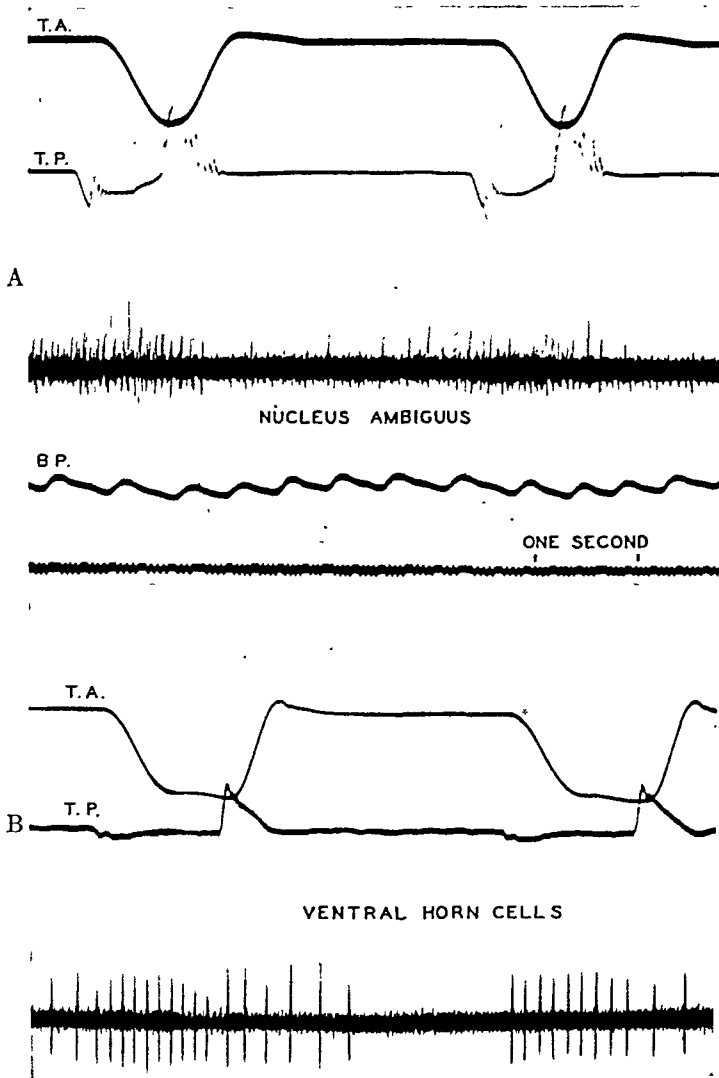


Fig. 7A. Inspiratory signals recorded from N. ambiguus and ventral horn cells drawn on the basis of effects produced by stimulation or removal of various regions. Our method differs substantially in that any portion of the central nervous system yielding respiratory signals is accepted as part of the complete respiratory mechanism. But only by inference and by confirmation do the findings throw light upon the relative rôle played by the

constituent parts. Attention has centered about the *alae cinereae*, the *fasciculus solitarius* and the *formatio reticularis*.

Schiff (1858) found that he could destroy many parts of the brain stem including Flourens's vital node without stopping respiration. When he destroyed the superior, lateral part of the *ala cinerea* he caused a cessation of respiration on the corresponding side of the body. Girard (1890) confirmed the results of Schiff. The destruction of the external part of the *ala cinerea* and the destruction of the posterior part of the nucleus of X on one side causes the cessation of respiration on that side. If the lesion is bilateral then the result is death. This region of the stem has, on the other hand, been destroyed by Gierke (1873) and Gad and Marinescu (1892) without affecting respiration. The finding of respiratory potentials in this region necessarily includes it as part of the respiratory mechanism.

Fasciculus solitarius. Gierke (1873) showed that destruction of the *alae cinereae* did not stop respiration. He checked the results of his lesions microscopically and came to the conclusion that the only part of the medulla which was essential for respiration was the *fasciculus solitarius*. This is often known as Gierke's *fasciculus*. Krause thought that this *fasciculus* connected the nucleus of the vagus with the phrenic nucleus. Girard (1890) considered the *fasciculus solitarius* as a descending respiratory path and not as a center. This portion of the brain stem was destroyed by Mislowsky, Gad and Marinescu and Bechterew. More recently Allen (1927) reported that destruction of all the terminal nuclei of the *tractus solitarius* in the spino-medullary region of the cat and guinea pig caused no permanent disturbance of respiration. Destruction of the trigemino-vagal portion of the nucleus *tractus solitarius* causes no more permanent change to respiration than severance of both vagi. Here again the finding of respiratory signals in this structure necessarily include it as part of the respiratory mechanism.

Reticular formation. In 1847, Longet destroyed the pyramids and the restiform bodies in the neighborhood of the vagus nucleus, without interfering with breathing, but "the isolated destruction of the '*faisceau intermediare du bulbe*', at the same level, caused instantaneous suspension of breathing." He added that this bundle contained numerous nerve cells and gray matter and for that reason appeared to be well suited to serve as a center of some particular function. According to Mislowsky (1885) this appears to be the medial reticular formation.

Since the time of Longet much work has been done on the relation of the reticular formation to respiration. In 1858 Flourens was forced to recognize his respiratory point as a bilateral structure, which was in the dorsal part of the reticular formation, in the neighborhood of the vagal nuclei, on both sides of the raphe just above the obex. Mislowsky (1885) considered the respiratory center as a double nucleus lying in the reticular formation

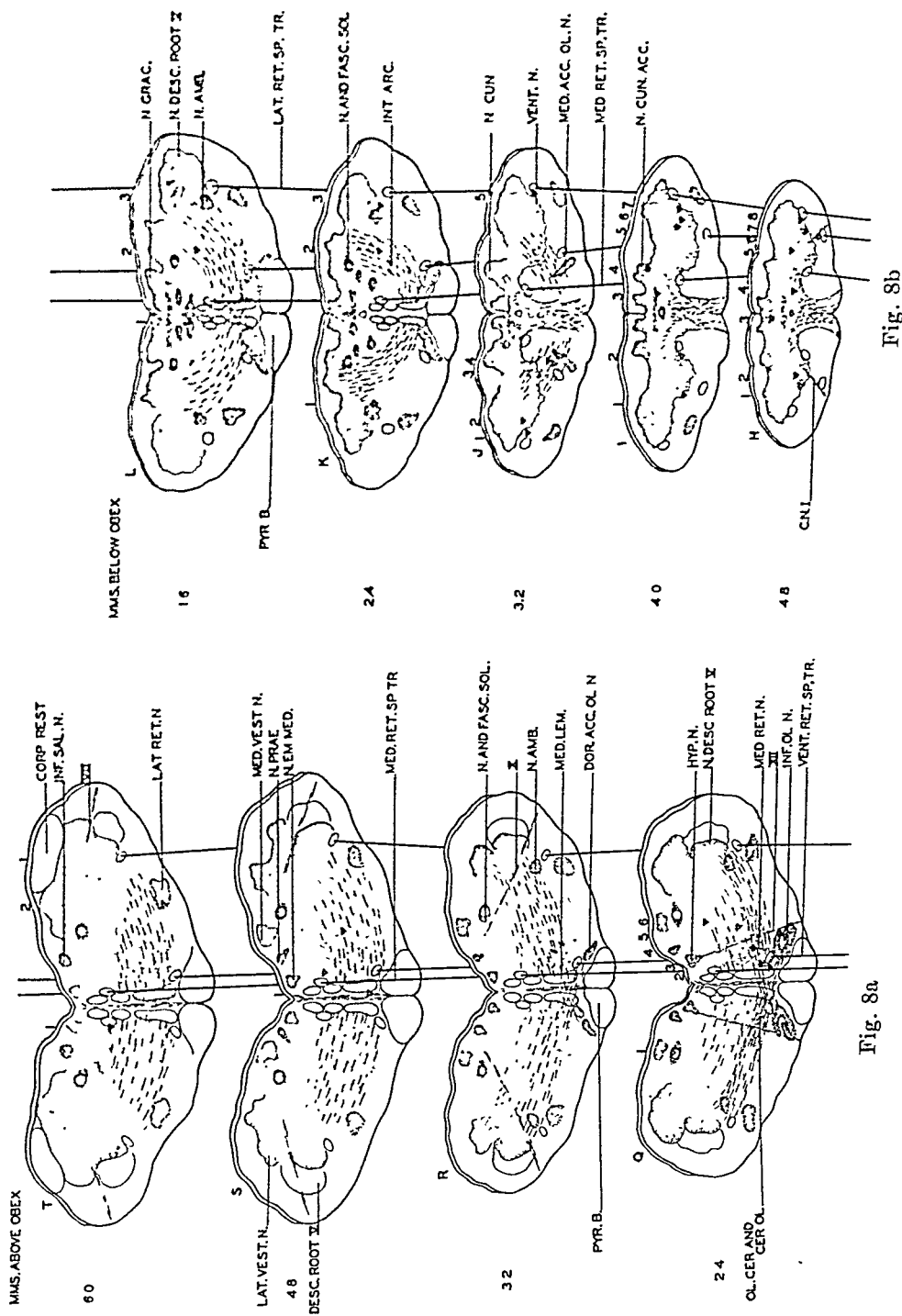


Fig. 8b

Fig. 8a

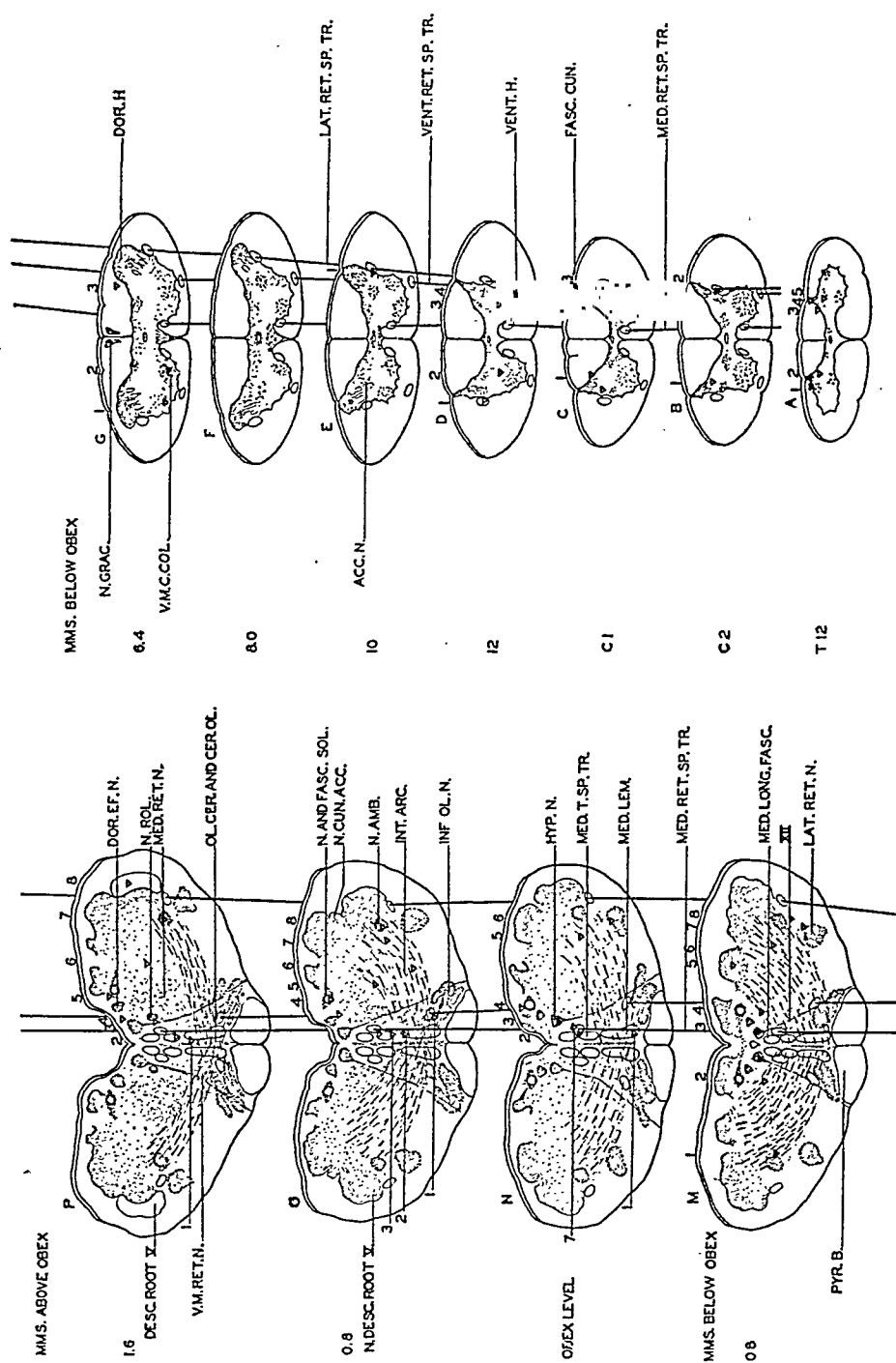


Fig. 9a

Fig. 9b

Figs. 8 and 9. Histological localization of potentials. Compare with table 1. See key for abbreviations.

on each side of the median raphe mainly internal to the roots of the hypoglossal nerves. This would correspond to the medial reticular formation. Destruction of this area by pricking abolished the respiratory movements immediately while stimulation of the area electrically resulted in a more rapid rate of respiration. Laborde (1890) found a small region on each side of the raphe and just above the point of the calamus which when destroyed for half the thickness of the medulla permanently stopped respiration. This perhaps corresponds to the medial reticular formation. He obtained no results from destruction of parts of the brain other than the medulla. Gad and Marinescu (1892) destroyed various parts of the brain stem with hot glass rods. They showed that only those lesions which were deep in the reticular formation stopped respiration. They could destroy Flouren's vital node, the external part of the alae cinereae and the solitary bundle without stopping respiration (regions of Flourens, Longet, Schiff, Gierke, and Mislawsky). These same workers stimulated deep in the stem with fine needles varnished except at the tips. When these needles were in the reticular formation there was an acceleration of respiratory rate and not a tetanization of the inspiratory muscles as follows stimulation of the lateral bundles of the cervical cord. In 1894 Arnheim found that stimulation between the nucleus of VI and the calamus accelerated respiration if the electrodes touched the reticular gray. Stimulation of the motor nucleus of VII caused only the muscles of the nose to respond, contrary to the opinion of Grossmann. Kohnstamm (1900) considered the reticular formation as an important regulatory mechanism for the nuclei supplying respiratory muscles. After section of the spinal cord in its upper part he obtained a degeneration of the reticular formation in the region Bechterew describes as his center. Bechterew (1908) states "The respiratory nucleus lies, as my own investigations show, only for the lesser part medial to the hypoglossal roots, for the greater part lateral to them. Each time I obtained, on transection or circumscribed destruction of the medulla oblongata, an arrest of respiration, in those cases when that small region of gray substance, which lies just above the calamus scriptorius chiefly lateral to the hypoglossal nucleus in the depths of the medulla oblongata, was destroyed." In 1931 Finley reported upon two cases of respiratory failure. His autopsies show congestion of the upper cervical cord, with round cell infiltration and partial destruction of the anterior horn cells and bilateral areas of necrosis in the reticular formation. From his review of the literature and from these two cases of respiratory failure he concludes that one of the main integrative levels for respiratory impulses lies in the reticular formation. Johnston (1932) found marked damage to the cells of the medullary and pontal centers in six cases of death from pneumonia. The sensory nuclei showed more damage than the motor nuclei. Cells of the reticular gray matter showed the most severe degenerative changes.

Keller (1928-29) from his studies of the polypnea mechanism concludes that the reticular formation is not important in the transmission of the impulses since extensive lesions of various kinds can be made in the medial part of the medulla and pons without affecting rapid respiration while lesions in the lateral regions stop the rapid respiration. According to Papez (1926) and Allen (1927), the reticulo-spinal tracts are employed to conduct the impulses to the motor cells of the respiratory muscles. According to Papez, the medial reticulo-spinal tract is the most extensive. It has its origin from large reticular cells of the pons and isthmus and from large cells of the upper medulla oblongata. The majority of the fibers of this tract remain on the same side, but a few cross and descend with fibers of the opposite side. The lateral reticulo-spinal tract has its origin from pontal reticular cells medial to the masticator nucleus, and from reticular cells near the mid-line extending to the genu of the facial nerve. The ventral reticulo-spinal tract in some instances appears to be a direct continuation of the descending limb of the brachium conjunctivum coming possibly from the nuclear masses around the brachium conjunctivum, but not all lesions in this region yield the tract. Other evidence indicates that it is crossed, decussating through Bechterew's reticular nucleus and having its origin in the ventro-lateral tegmental nucleus just lateral to the medial longitudinal bundle. It then follows along the dorsal accessory olive into the ventral funiculus of the cord.

Experimental findings indicating that the reticular formation serves as the main station for integration of the respiratory signals are amply confirmed by our electrical soundings. No other structures yielded respiratory potentials in such abundance or with such certainty and the tapping of potentials from the reticulo-spinal tract is further strong evidence of the integrating rôle of the reticular system. The frequent occurrence of potentials in the lateral reticular nuclei marks a more lateral extension of the respiratory mechanism in the formatio reticularis than that recently described by Henderson and Craigie (1936) if our interpretation of their measurements is correct. Through localized sectioning of fiber paths, Allen concludes that the ventral and lateral reticulo-spinal tracts are the main channels conducting impulses from the cerebrum and superior colliculus to the respiratory nuclei of the spinal cord. Our findings confirm this view and add the medial reticulo-spinal tract as an accessory highway. Furthermore, our findings indicate that the normal pathways of respiratory impulses are similar to those of modified breathing produced by stimulation of higher centers (Allen 1927).

Of no little interest are the five sets of potentials encountered in our total of twenty experiments on higher "respiratory centers." Of the five sources of potentials four were localized and one lesion was lost (see the upper four locations in fig. 1A). One lesion involved the medial reticulo-spinal formation, internal arcuate fibers, and possibly the medial reticulo-spinal

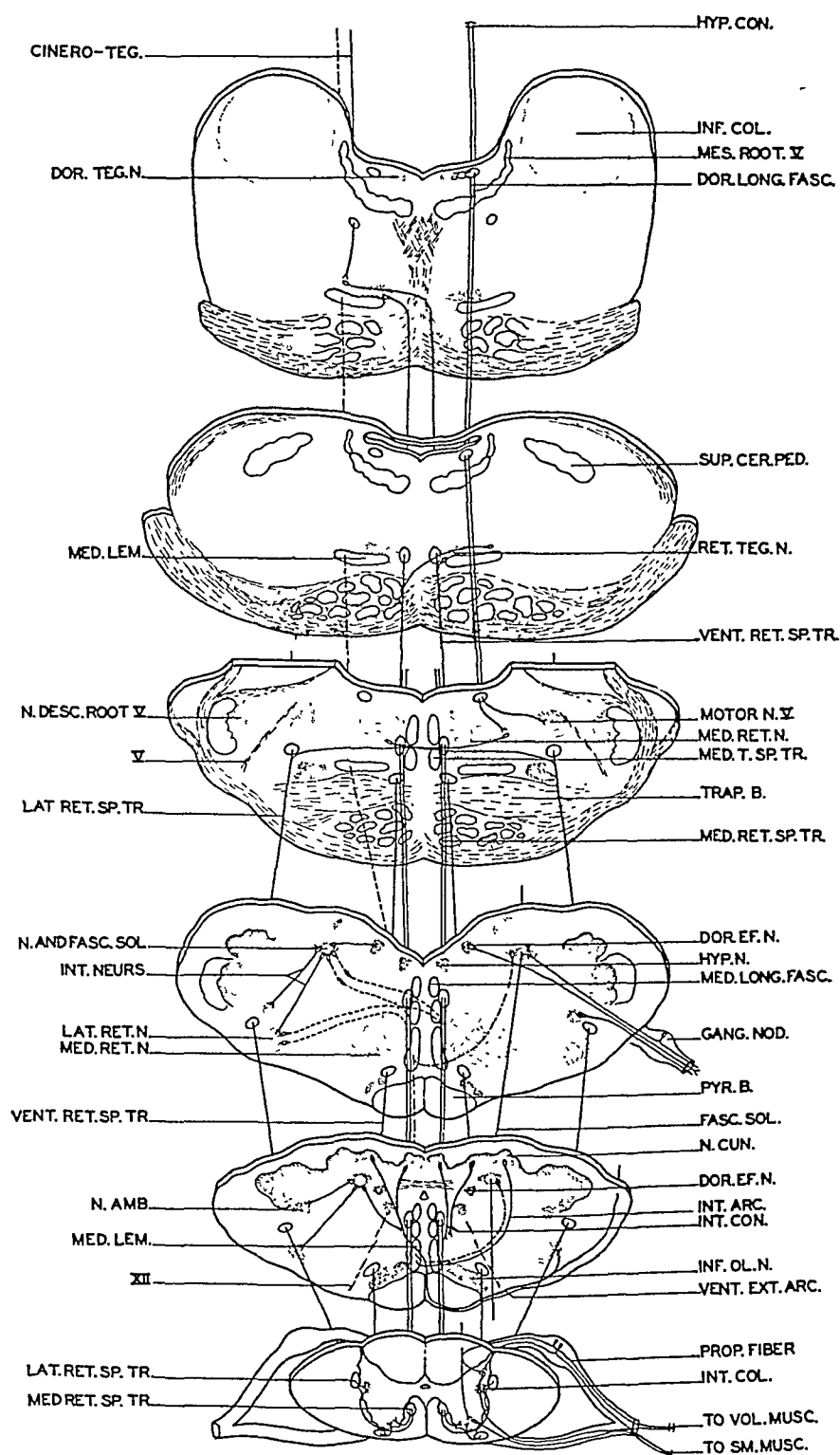


Fig. 10. Schema of central respiratory mechanism. See key for abbreviation.

tract. The remaining three involved the brachium conjunctivum. Thus they suggest a contributing control through the cerebellum despite our failure to find respiratory signals in a preliminary exploration of that structure, and furthermore, they support Papez's suggestion regarding the brachium conjunctivum as a source of fibers in the ventral reticulospinal tract.

DISCUSSION. Bearing in mind the destructive effects of pin prick lesions, such as described by Flourens, we were surprisingly encouraged in the possibilities of our procedure during our early experiments on the vital region of the medulla. Dense riddling of the entire lower medulla in some animals seemed to have no effects whatever on breathing and permitted extensive observations. To be sure, the animals deteriorated, some very slowly and others rapidly, but previous decortication seemed more related to collapse than did sounding for potentials. The fineness of our electrodes could easily account for the lack of damage but repeated galvanic lesions seemed to produce only momentary effects while the current was flowing. This immunity to damage is probably related to the structural and func-

Acc. N.—Spinal accessory nucleus; *Cinero-Teg.*—Cinero-tegmental connection; *C. N. I.*—Cervical nerve I; *Corp. Rest.*—Corpus restiforme; *Desc. Root V*—Descending root of trigeminal; *Dor. Acc. Ol. N.*—Dorsal accessory olivary nucleus; *Dor. Ef. N.*—Dorsal efferent nucleus; *Dor. H.*—Dorsal horn; *Dor. Long. Fasc.*—Dorsal longitudinal fasciculus; *Dor. Teg. N.*—Dorsal tegmental nucleus; *Fasc. Cun.*—Fasciculus cuneatus; *Fasc. Sol.*—Fasciculus solitarius; *Gang. Nod.*—Ganglion nodosum; *Hyp. Con.*—Hypothalamic connection; *Hyp. N.*—Hypothalamic nucleus; *Inf. Col.*—Inferior colliculus; *Inf. Ol. N.*—Inferior olivary nucleus; *Inf. Sal. N.*—Inferior salivary nucleus; *Int. Arc.*—Internal arcuate fibers; *Int. Con.*—Internuncial connections between gracile and cuneate nuclei and medial reticular formation; *Int. Col.*—Intermediolateral cell column; *Int. Neurs.*—Internuncial neurones; *Lat. Ret. N.*—Lateral reticular nucleus of the medulla oblongata; *Lat. Ret. Sp. Tr.*—Lateral reticulospinal tract; *Lat. Vest. N.*—Lateral vestibular nucleus; *Med. Acc. Ol. N.*—Medial accessory olivary nucleus; *Med. Lem.*—Medial lemniscus; *Med. Long. Fasc.*—Medial longitudinal fasciculus; *Med. Ret. N.*—Medial reticular nucleus of the medulla oblongata; *Med. Ret. Sp. Tr.*—Medial reticulospinal tract; *Med. T. Sp. Tr.*—Medial tecto-spinal tract; *Med. Vest. N.*—Medial vestibular nucleus; *Mes. Root V*—Mesencephalic root of trigeminal nerve; *Motor N. V.*—Motor nucleus of trigeminal nerve; *N. Amb.*—Nucleus ambiguus; *N. and Fasc. Sol.*—Nucleus and fasciculus solitarius; *N. Fasc. Sol.*—Nucleus fasciculus solitarius; *N. Cun.*—Nucleus cuneatus; *N. Cun. Acc.*—Nucleus cuneatus accessorius; *N. Desc. Root V*—Nucleus descending root of trigeminal nerve; *N. Em. Med.*—Nucleus eminentia medialis; *N. Grac.*—Nucleus gracilis; *N. Prae.*—Nucleus praeposticus; *N. Rol.*—Nucleus of Roller; *Ol. Cer. and Cer. Ol.*—Olivocerebellar and cerebello-olivary fibers; *Prop. Fiber*—Proprioceptive fiber; *Pyr. B.*—Pyramidal bundle; *Ret. Teg. N.*—Reticular tegmental nucleus of Bechterew; *Sm. Musc.*—Smooth, involuntary muscle; *Sup. Cer. Ped.*—Superior cerebellar peduncle; *Trap. B.*—Trapezoid body; *Vent. Ext. Arc.*—Ventral external arcuate fibers; *Vent. H.*—Ventral horn; *Vent. N.*—Ventral-lateral nucleus; *Vent. Ret. Sp. Tr.*—Ventral reticulospinal tract; *V. M. C. Col.*—Ventral-medial cell column; *V. M. Ret. N.*—Ventral-medial reticular nucleus; *Vol. Musc.*—Striated, voluntary muscle.

tional organization of the reticular formation. The impression we have gained is that the potentials are relatively few and scattered and that the anatomical description "scattered reticular gray matter" is descriptive of its function as well. The relative scarcity of reticular potentials, the large size and spacing of the cells, considered with the copious innervation of muscles as represented in the ventral column of the cord suggest a multiple innervation of the ventral horn cells with a high step-up ratio.

The fact that potentials vary considerably in rate, and that they may vanish and reappear under conditions modifying the respiratory act, indicates that the mechanisms of recruitment and of adjustment of rhythm are at the disposal of the reticular formation. We would venture to say, however, that the acceleration of reticular firing during inspiration is not equal to that of the motor nerve cells.

The higher incidence of inspiratory potentials compared with expiratory (3 to 1 ratio) agrees with the greater incidence of contraction of inspiratory muscles noted in systematic electric exploration of respiratory muscles (Gesell, 1936a).

The absence of a systematic or localized arrangement of inspiratory and expiratory potentials, as plotted in figure 1A, indicates a lack of grouping of cells into inspiratory and expiratory nuclei and changing potentials with progressive penetration of the electrodes yields the same conclusion. This point is illustrated by an example of a single sounding of 3 mm. extent, which successively tapped-off seven sets of potentials in the order given—inspiratory, expiratory, expiratory (new set), regular frequency without respiratory rhythm, expiratory, inspiratory, and expiratory. The previous conceptions of compact, definitely localized inspiratory and expiratory centers, held by some investigators, probably should be revised in the light of our findings. We have no evidence of special groupings of potentials favoring Lumsden's views.

The great abundance of potentials of continuing regular frequency was a uniform finding and has raised the question of the source of the drive perpetuating these signals. We have seen an instance in which such signals were modified to take on a respiratory rhythm (fig. 5B), but have no evidence to ascertain the destination of these signals. They may be signals on their way to striated muscle or to the involuntary muscle of heart, blood vessels or lungs. Their relatively low source in the medulla probably favors the first view. Granting that, it is reasonable, in a steady state of anesthesia, to expect a steady reflex drive through the proprioceptive and cerebellar route for muscles in fixed position. Should any of these muscles be called to contribute towards pulmonary ventilation (as in fig. 5) steady fire would give way to interruption corresponding to respiratory rhythm.

An attempt to construct a schema of the central respiratory mechanism in our state of knowledge is an invitation to error and incompleteness but a few tentative reflections may not be amiss. It would seem: that the

integrative function of the reticular formation for respiratory movements is more definitely established by our findings; that we are privileged to look upon this formation as the part about which the incoming and outgoing respiratory highways are constructed; that the reticular gray matter is influenced by every type of sensory signal, but most importantly through the fifth, ninth, tenth, eleventh, and twelfth cranial nerves and the sensory roots of the cord; that signals entering by these channels may reach the reticular gray by various paths (see schema) some direct, others by circuitous routes; that removal of the reticular gray matter would remove all adequate means of integrating respiratory signals and leave no organization for concerted orderly recruitment of respiratory muscles and therefore lead to a respiratory death; that the reticular gray directs its signals in orderly fashion down the reticulo-spinal tracts at the end of which the respiratory motor neurones are fired; that a mechanism capable of further control may exist at this point of firing.

Our experiments offer no entering wedge to open the question of automaticity of reticular gray matter.

In conclusion we feel that our present experiments are of a preliminary nature, that they offer mainly a skeleton of the respiratory mechanism, on which we hope to build a more complete picture of respiratory signals under conditions modifying the respiratory act.

SUMMARY AND CONCLUSIONS

The structure of the central mechanism controlling breathing and the function of its constituent parts were studied with a method of electrical sounding. The central gray axis stem of the dog including the diencephalon, mesencephalon, pons, medulla, and upper cervical cord were systematically explored with fine needle electrodes bared at the tips. Potentials showing a respiratory rhythm were photographed and their sources histologically localized.

Respiratory potentials were found in greatest abundance and with greatest certainty in the region about the obex. The intimate relation of these potentials to important respiratory nuclei was graphically presented.

Explorations of the mesencephalon and diencephalon in twenty experiments revealed only five sets of respiratory potentials, indicating that the main "center" or controlling mechanism resides in the medulla oblongata and that impulses coming from above are primarily of a regulative nature.

Exploration of the cord showed extensive respiratory potentials. This finding conforms with the necessary chain of impulses for the innervation of respiratory muscles.

The upper limit of the medullary respiratory potentials cuts across the upper quarter of the N. hypoglossus, N. ambiguus, and N. fasciculus solitarius. The incidence of respiratory potentials extending continuously

from the medulla through the cord prevents similar establishment of the lower border of the medullary respiratory mechanism, but a line marking the disappearance of reticular formation potentials places the lower border at the cephalic edge of the pyramidal decussation. These limits are in agreement with findings of many workers localizing the respiratory center in the medulla oblongata.

Lesions at the sites of respiratory potentials involved a large variety of structures indicating the diversity of highways for respiratory signals. These structures and their associated potentials are tabulated in detail.

The most common source of respiratory signals was in the reticular formation. It is concluded that these formations are intermediary stations interposed between incoming and outgoing signals and that they are essential to the respiratory act.

The incidence of inspiratory potentials, like that in respiratory muscles, was considerably greater than the incidence of expiratory potentials.

There was no anatomical grouping of signals indicating a structural collection of cells as demanded by previously postulated compact inspiratory and expiratory centers.

The occurrence of potentials in the brachium conjunctivum suggests a contribution of the cerebellum to the integration of the respiratory act.

REFERENCES

- ADRIAN, E. D. *J. Physiol.* **79**: 332, 1933.
 ADRIAN, E. D. AND D. W. BRONK. *J. Physiol.* **66**: 81, 1928.
 ADUCCO, V. *Arch. ital. de biol.* **13**: 89, 1890.
 ALLEN, W. F. *J. Comp. Neurol.* **43**: 451, 1927.
 ARNHEIM, R. *Arch. f. Anat. u. Physiol.* **1**: 1894.
 ASHER, B. *Berliner klin. Wehnschr.* **53**: 772, 1916.
 ASHER, L. AND F. LUSCHER. *Ztschr. f. Biol.* **38**: 499, 1899.
 BARCROFT, J. *The architecture of physiological function.* Cambridge, 1934.
 BAZETT, H. C. AND W. G. PENFIELD. *Brain* **45**: 185, 1922.
 BECHTEREW, W. *Arch. f. Anat. u. Physiol.* **500**, 1899.
 Die Functionen der Nervencentra. Jena, 1908.
 BELL, C. *The nervous system of the human body.* Washington, 1833.
 BROWN, T. G. *J. Physiol.* **48**: xxxii, 1914.
 CHRISTIANI, A. *Arch. f. Anat. u. Physiol.* **295**, 1880.
 Zur Physiologie des Gehirns. Berlin, 1885.
 COOMBS, H. C. *This Journal* **56**: 459, 1918.
 This Journal **50**: 511, 1920.
 Science **71**: 136, 1930.
 COOMBS, H. C. AND F. H. PIKE. *This Journal* **45**: 569, 1918.
 CORDIER, D. AND C. HEYMANS. *Ann. de physiol. et des physicochimie biol.* **11**: 535, 1935.
 ECTORS, L. AND N. L. BROOKENS. *Proc. Am. Physiol. Soc.* (in press) 1936.
 FERRIER, D. *The functions of the brain.* London, 1876.
 FINLEY, K. H. *Arch. Neurol. and Psychiat.* **26**: 754, 1931.

- FLOURENS, J. P. M. *Recherches exp. sur les prop. et les fonctions du system nerveux.* Paris, 1842.
 Compt. rend. Acad. de sc. **33**: 437, 1851.
 Compt. rend. Acad. de sc. **47**: 803, 1858.
- FRANCK, F. *Arch. de physiol. norm. et pathol.* **2**: 546, 1892.
- FREDERICQ, L. *Arch. f. Anat. u. Physiol. Suppl. Band.* **51**, 1883.
- GAD, J. AND G. MARINESCU. *Compt. rend. Acad. de sc.* **115**: 444, 1892.
- GALEN. *De Anat. Administr. lib. VIII, cap. IX.*
- GESELL, R. *This Journal Proc.* **105**: 37, 1933.
This Journal **115**: 168, 1936a.
This Journal **116**: 228, 1936b.
- GIERKE, H. *Pflüger's Arch.* **7**: 583, 1873.
- GIRARD, H. *Mem. Soc. phys. et hist. nat. Geneve. Vol. Suppl.* 1890.
- GROSSMANN, M. *Sitzungsber. d. Akad. Wien 3 Abth.* **98**: 385, 1889.
- HENDERSON, V. E. AND T. A. SWEET. *This Journal* **91**: 94, 1929.
- HENDERSON, V. E. AND E. H. CRAIGIE. *This Journal* **115**: 520, 1936.
- HESS, L. AND E. POLLAK. *Wien. Arch. f. inn. Med.* **14**: 435, 1927.
- HUBER, G. C. *Contributions to medical science, Dedicated to Alfred Scott Warthin.* George Wahr, Ann Arbor, 1927, p. 1.
- HUBER, G. C. AND E. C. CROSBY. *Arch. Neurol. and Psychiat.* **22**: 187, 1929.
- HYDE, I. H. *J. Morph.* **9**: 347, 1894.
This Journal **10**: 236, 1904.
- JOHNSTON, J. M. *Arch. Path.* **14**: 461, 1932.
- JOUKOWSKI. *J. de physiol. et de path. gen.* **1**: 575, 1899.
- KARPLUS, J. P. AND A. KREIDL. *Pflüger's Arch.* **129**: 138, 1909.
Pflüger's Arch. **135**: 401, 1910.
- KELLER, A. D. *Proc. Soc. Exper. Biol. and Med.* **25**: 266, 1928.
This Journal **89**: 289, 1929.
This Journal **96**: 59, 1931.
- KELLER, A. D. AND W. K. HARE. *Proc. Soc. Exper. Biol. and Med.* **29**: 1069, 1932.
- KOHNSTAMM, O. *Monatschr. f. Psychiat. u. Neurol.* **161**, 1900.
- KNOLL, P. *Sitzungsber. d. Akad. Wien. 3 Abth.* **92**: 328, 1885.
- KRONECKER, H. *Deutsch. med. Wehnschr.* 785, 1887.
- KRONECKER, H. AND M. MARCKWALD. *Arch. f. Anat. u. Physiol.* 441, 1880.
- LABORDE, J. *Compt. rend. Soc. de biol. neuvieme series* **2**: 620, 1890.
- LANGLOIS, J. P. AND L. GARRELON. *Compt. rend. Soc. de Biol.* **65**: 715, 1908.
- LEGALLOIS, C. J. *Experiences sur le principe de la vie.* Paris, 1812.
- LEITER, L. AND R. R. GRINKLER. *Arch. Neurol. and Psychiat.* **31**: 54, 1934.
- LEWANDOWSKY, M. *Arch. f. Anat. u. Physiol.* 785, 1881.
Arch. f. Anat. u. Physiol. 195, 1896.
Arch. f. Anat. u. Physiol. 483, 1896.
- LOEWY, A. *Pflüger's Arch.* **42**: 245, 1888.
- LONGET, A. *Arch. gen. de med.* 4th series **13**: 374, 1847.
Traite de Physiologie III, 1868.
- LORRY, A. C. *Acad. roy. des sc. Mem. de math. et de physique* **3**: 344, 1760.
- LOTMAR, F. *Monogr. a. d. gesamtgebiete d. Neurol. u. Psychiat.* Heft 48, 1926.
- LUMSDEN, T. *J. Physiol.* **57**: 153, 1923.
J. Physiol. **57**: 354, 1923.
J. Physiol. **58**: 81, 1923.
J. Physiol. **58**: 111, 1923.
- MACLEOD, J. J. R. *Trans. Roy. Soc. of Canada sect. 5*, 85, 1919.
- MACLEOD, J. J. R. AND S. U. PAGE. *This Journal* **60**: 134, 1922.

- MARCKWALD, M. *Ztschr. f. Biol.* 23: 149, 1887.
- MARTIN, H. N. *J. Physiol.* 1: 131, 1878.
- MARTIN, H. N. AND W. B. BOOKER. *J. Physiol.* 1: 370, 1878-79.
- MINGAZZINI, G. *Hand. d. mikr. anat. d. Menschen* 4: 579, 1928.
- MISLAWSKY, N. *Centralbl. f. d. med. Wissensch.* 23: 465, 1885.
- NICHOLSON, H. C. *This Journal* 115: 402, 1936a.
- Proc. Am. Physiol. Soc. (in press), 1936b.
- NIKOLAIDES, R. *Arch. f. Anat. u. Physiol.* 465, 1905.
- NONNE, M. *Verh. d. deutsch. ges. f. inn. Med.* 67: 1923.
- PAPEZ, J. W. *J. Comp. Neurol.* 41: 365, 1926.
- PETTE, H. *Deutsch. Ztschr. f. Nervenheilk.* 76: 1, 1923.
- RANSON, S. W. AND H. W. MAGOUN. *Arch. Neurol. and Psychiat.* 29: 1179, 1933.
- RANSON, S. W., H. KABAT AND H. W. MAGOUN. *Arch. Neurol. and Psychiat.* 33: 467, 1935.
- SACHS, E. *J. Exper. Med.* 14: 408, 1911.
- SCHIFF, J. M. *Lehrbuch der Physiologie des Menschen. Lahr*, 1858-59.
- SCHOEN, R. *Arch. f. Exper. Path. u. Pharmacol.* 135: 155, 1928.
- SMITH, W. A. *Arch. Neurol. and Psychiat.* 15: 617, 1926.
- SPIEGEL, E. A. AND H. ENGHOFF. *Ztschr. f. d. ges. exper. med.* 47: 193, 1925.
- SPRINGER, M. G. *Arch. Neurol. and Psychiat.* 19: 834, 1928.
- STERN, F. *Die epidemische Encephalitis.* Berlin, 1923.
- TEREGULOW, A. G. *Pflüger's Arch.* 221: 486, 1929.
- TRENDELENBURG, W. *Monatsch. f. Psychiat. u. Neurol.* 28: 186, 1910. *Pflüger's Arch.* 135: 467, 1910.
- TREVAN, J. W. *J. Physiol.* 50: xliii, 1916.
- TREVAN, J. AND E. BOOCK. *J. Physiol.* 56: 331, 1922.

THE EFFECT OF INTRAVENOUS ADMINISTRATION OF PROTAMINE INSULIN

BERNARD B. LONGWELL AND ABE RAVIN

*From the Departments of Biochemistry and Medicine, University of Colorado School of
Medicine, Denver*

Received for publication June 15, 1936

During some clinical investigations of the effects of protamine insulin compound (protamine insulin, Lilly¹) which are now being carried out under the direction of Dr. James J. Waring, it seemed desirable to investigate some of the properties of the compound on experimental animals in which the results would not be complicated by the presence of disease. Hagedorn, Jensen, Krarup and Wodstrup (1) first reported the development of protamine insulinate. They found that the material was absorbed from the site of injection more slowly than ordinary insulin, a fact which has been confirmed by direct observations on rabbits by Beecher and Krogh (2). Slower absorption results in a prolongation of activity which makes it possible to treat patients with smaller doses of insulin. Furthermore, the diurnal fluctuations in the blood sugar become less pronounced and thereby are made to assume a condition more nearly like that of the normal individual. These latter findings have been confirmed by Root, White, Marble and Stotz (3), by Sprague, Blum, Osterberg, Kepler and Wilder (4), and by Kerr, Best, Campbell and Fletcher (5).

METHODS. The experiments were carried out upon female albino rabbits. Females were chosen because of the report of Dotti (6) that the female is more constant than the male in its response to insulin. The weights of the animals ranged from 1500 to 2400 grams. No special diets were used; the rabbits were maintained on the stock colony diet. Each animal was subjected to a 24 hour fast before administration of the insulin or protamine insulin. The dose used was 1.5 unit per kilogram of body weight.

Blood was withdrawn from the ear vein before administration of the insulin or protamine insulin and at $\frac{1}{2}$, 1, 2 and 3 hours thereafter. Blood sugar was determined by the method of Folin and Wu (7) modified for use with the Peebles-Lewis colorimeter (8). This method gives values for the total reducing substance of the blood.

¹ The protamine insulin compound was furnished by Eli Lilly & Co., Indianapolis, whose coöperation we gratefully acknowledge.

TABLE 1

Comparison of the effects of insulin and protamine insulin after intravenous administration

RABBIT NUMBER	BLOOD SUGAR, MGM. PER 100 CC.				
	Before insulin	$\frac{1}{2}$ hour	1 hour	2 hours	3 hours
Protamine insulin					
1	<i>112</i>	<i>72</i>	<i>60</i>	<i>76</i>	<i>98</i>
2	<i>108</i>	<i>67</i>	<i>63</i>	<i>90</i>	<i>116</i>
3	<i>Lost</i>	<i>78</i>	<i>55</i>	<i>70</i>	<i>114</i>
4	<i>106</i>	<i>60</i>	<i>28</i>	<i>60</i>	<i>94</i>
5	<i>98</i>	<i>60</i>	<i>47</i>	<i>64</i>	<i>80</i>
6	<i>116</i>	<i>62</i>	<i>60</i>	<i>72</i>	<i>96</i>
7	<i>104</i>	<i>59</i>	<i>31</i>	<i>69</i>	<i>98</i>
Protamine insulin and regular insulin*					
8	<i>114</i>	<i>72</i>	<i>43</i>	<i>40</i>	<i>60</i>
	105	57	32	38	76
9	<i>88</i>	<i>78</i>	<i>50</i>	<i>88</i>	<i>106</i>
	109	88	56	92	113
10	<i>90</i>	<i>68</i>	<i>47</i>	<i>53</i>	<i>88</i>
	90	55	39	49	68
11	<i>92</i>	<i>60</i>	<i>44</i>	<i>58</i>	<i>89</i>
	120	58	45	77	120
12	<i>94</i>	<i>62</i>	<i>48</i>	<i>56</i>	<i>65</i>
	112	50	44	53	98
13	<i>78</i>	<i>63</i>	<i>51</i>	<i>66</i>	<i>86</i>
	109	54	55	72	99
14	<i>130</i>	<i>68</i>	<i>43</i>	<i>67</i>	<i>102</i>
	118	47	40	84	120
15	<i>110</i>	<i>91</i>	<i>45</i>	<i>57</i>	<i>104</i>
	119	89	57	76	120
16	<i>100</i>	<i>67</i>	<i>40</i>	<i>60</i>	<i>90</i>
	118	66	48	60	98

* Protamine insulin in italics.

Nine animals were given protamine insulin subcutaneously. Sixteen additional animals received protamine insulin intravenously. To nine of these latter sixteen animals regular insulin was given intravenously in

order that we might compare the results. The animals received protamine insulin first and, after a rest period of at least five days, they were given the same dose of regular insulin.

RESULTS. In confirmation of the results obtained with diabetic patients, our results with rabbits show that after subcutaneous injection the effect of protamine insulin is greatly prolonged as compared to the response of the blood sugar to regular insulin. Some of the results in which there was a fairly rapid action together with a prolongation of the effect suggested to us that we might have inadvertently injected some of the material into a small blood vessel as well as under the skin. We carried out experiments to determine the effect of intravenous administration of protamine insulin, and the results of these experiments are recorded in table 1. The results show that there is no essential difference between the reaction of the same animal to the insulin and protamine insulin when given intravenously. The low solubility of protamine insulin at the pH of the body appears to affect the activity of the compound only so far as its absorption from a subcutaneous injection is concerned. When it is directly dispersed in the blood, it acts in a manner practically identical with that of ordinary insulin.

The failure to obtain delayed reaction after the intravenous administration of protamine insulin is further evidence that the prolonged activity of the compound which follows subcutaneous injection depends upon its delayed absorption. Protamine insulin is a particulate suspension which is least soluble at pH 7.3 (1). When this type of substance is administered subcutaneously it is deposited in a restricted area in the tissue spaces, and it has available only a small amount of slowly moving solvent fluid. This probably accounts for the fact that its absorption into the blood stream is slow and difficult. However, when it is injected into the blood it is rapidly distributed into a large amount of solvent. Under these latter circumstances the factor of low solubility at the existing pH does not retard solution of the compound to the point where there is an appreciable delay in its action. For this reason the blood sugar responds promptly to the intravenous administration of protamine insulin.

SUMMARY

The prolonged effect of protamine insulin after subcutaneous injection has been confirmed. The action of the compound after intravenous injection, however, does not differ significantly from the action of regular insulin similarly injected.

REFERENCES

- (1) HAGEDORN, H. C., B. N. JENSEN, N. B. KRARUP AND I. WODSTRUP. *J. A. M. A.* 106: 177, 1936.
- (2) BEECHER, H. K. AND A. KROGH. *Nature* 137: 458, 1936.

- (3) ROOT, H. F., P. WHITE, A. MARBLE AND E. H. STOTZ. J. A. M. A. 106: 180, 1936.
- (4) SPRAGUE, R. G., B. B. BLUM, A. F. OSTERBERG, E. J. KEPLER AND R. M. WILDER.
J. A. M. A. 106: 1701, 1936.
- (5) KERR, R. B., C. H. BEST, W. R. CAMPBELL AND A. A. FLETCHER. Canad. M. A.
J. 34: 400, 1936.
- (6) DOTTI, L. B. This Journal 114: 538, 1936.
- (7) FOLIN, O. AND H. WU. J. Biol. Chem. 38: 81, 1919.
- (8) PEEBLES, A. R. AND R. C. LEWIS. J. A. M. A. 70: 679, 1918.
LEWIS, R. C. J. Lab. and Clin. Med. 16: 914, 1931.

THE MECHANISM OF THE INHIBITORY ACTION OF VASODILATOR NERVES

EMIL BOZLER

*From the Eldridge Reeves Johnson Foundation for Medical Physics, University of
|Pennsylvania*

Received for publication June 15, 1936

The regulation of the tone of blood vessels is to a large extent effected by two antagonistic kinds of nerve fibers, vasoconstrictor and vasodilator. Impulses discharged through sympathetic nerves produce contraction of the muscular coat of the vessels and keep them normally in a condition of moderate constriction. Dilatation, on the other hand, which is due to a lengthening of the circular muscle fibers, can in the intact animal result from a cessation or diminution of vasoconstrictor activity. There is, however, a second mechanism for producing vasodilatation, and that is the action of specific inhibitory nerves. The latter mechanism, which alone is of interest in this work, is one of the most important cases of peripheral inhibition.

At least two hypotheses have been offered to explain this phenomenon. It has been suggested that the stimulation of inhibitory fibers produces a depression of the contractile mechanism. Other authors have assumed that the inhibition is an active response by means of which the muscle fibers can be set at a new and greater length. However, little experimental work has been done to verify these hypotheses. In the experiments which are reported here I have attempted to study this problem by influencing the muscular coat of blood vessels simultaneously by excitatory and inhibitory impulses and by a quantitative study of the interaction of their effects.

MATERIAL AND METHOD. In order to avoid the complications of a normal circulation it seemed advisable to use a perfusion method. The reactions of the blood vessels of a perfused preparation can be conveniently studied by recording the rate of flow of the perfusion fluid. It is well known that the largest part of the resistance to flow is located in the arterial system, so that changes in the rate of flow indicate with fair accuracy the changes in width of the peripheral vessels, mainly those of the arterioles, of the perfused organ.

The hind leg of the frog proved to be very suitable for these experiments. The rate of flow of the perfusion fluid going into the preparation

was measured by a sensitive flowmeter. Constriction of the blood vessels was produced by stimulating the sympathetic trunk; purely inhibitory effects were obtained by stimulating the dorsal roots.

The flowmeter which was used in these experiments is based on the principle of the Pitot tube (fig. 1). The perfusion fluid (Ringer's solution containing 0.65 per cent NaCl, 0.02 per cent KCl and 0.015 per cent CaCl_2 adjusted to pH 7.5 by phosphate buffer) passes through a capillary, *C*, the pressure difference across which is recorded by the differential manometer, *DM*. This manometer consists of two thin rubber membranes about 4 mm. in diameter connected together by a piece of metal, *X*, whose movements are transmitted by the torsion spring, *T*, and are recorded by a small mirror. The deflection of the meter is accurately

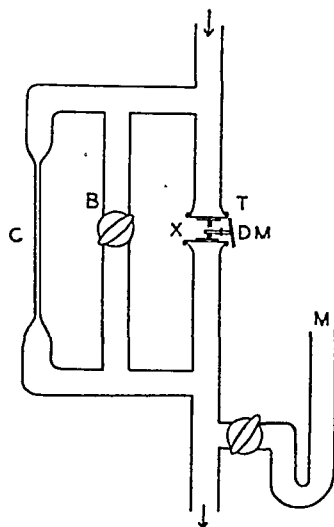


Fig. 1. Diagram of flow meter. *C*, capillary tube; *B*, bypass; *DM*, differential manometer; *M*, U-tube manometer; *T*, torsion spring; *X*, metal piece connecting the two rubber membranes.

proportional to the rate of flow. In the experiments which will be reported 1 mm. deflection on the recording camera corresponded to a change of flow of about 0.25 cmm. per second; this is a change of 1 to 2 per cent of the maximal rate of flow through the preparation.¹

¹ It should be noted that the pressure difference recorded by the differential manometer is only accurately proportional to the rate of flow at constant velocity. If the diameter of the vessels diminishes rapidly, a pressure will be produced by this contraction. A new stationary state will be established after some time which depends on the resistance to flow on both sides of the constricted vessel. The question whether this and the lag of the flowmeter introduces an appreciable distortion of the records of the contractions can be empirically tested by rapidly compressing the limb. It was found that a new constant rate of flow is established in a fraction of a second. The lag of the recording system is therefore small compared with the time relations of the contractions of the blood vessels.

The manometer, *M*, which is disconnected while the flow is recorded, measures the perfusion pressure; it was 10 to 12 cm. water in the experiments. For greatest sensitivity this pressure should be about one-half of the total pressure difference between the bottle containing the perfusion fluid and the preparation. This can be adjusted by choosing a capillary of suitable width. For most purposes the sensitivity so obtained is too great and wider capillaries can be used.

Drugs are applied by injection into the rubber connection near the preparation. During the injection the manometer, *M*, is connected to the flowmeter. This provides an outlet for the fluid and prevents the production of an excessive pressure by the injection.

Only *Rana pipiens* proved to be suitable for the experiments. The peculiar observation was made that in bullfrogs (*Rana catesbiana*) stimulation of the dorsal roots regularly produced vasoconstriction if the vessels were dilated, dilatation if the vessels were strongly constricted. This is presumably due to the presence of sympathetic fibers in the dorsal roots in this species. This observation is in line with older findings of the occasional occurrence in frogs of dorsal root fibers supplying the urinary bladder (Langley and Orbeli, 1910, review on this question: Brücke, 1927). In *Rana pipiens*, on the other hand, slight constrictor effects were observed only rarely on stimulation of the dorsal roots.

The animals were narcotised with ether, decapitated and eviscerated. The aorta was preserved and the cannula inserted into one of the femoral arteries so that only one leg was perfused. The sympathetic trunk of the perfused side was dissected out, leaving the rami to the sciatic nerve intact. One pair of stimulating electrodes was placed on the sympathetic trunk as close to the sciatic plexus as is possible without spread of the stimulating current. For the preparation of the dorsal roots the spinal canal was opened ventrally and all nerves except the dorsal roots supplying the sciatic nerve of the perfused leg were cut. The stimuli were usually applied to the whole caudal part of the spinal cord, although in some cases the cord was removed and the stimulating electrodes were attached to the roots directly. The nerves were stimulated by condenser discharges. A maximal stimulus was obtained by a condenser of 0.3μ F and 6 to 8 volts. To minimize polarization at the electrodes during the longer periods of stimulation a commutator was used to reverse the direction of alternate shocks. The dorsal roots were always stimulated at a frequency of about 20 shocks per second, the sympathetic fibers at various rates.

RESULTS. Figure 2A is an example of the records obtained with the method just described. A constriction of the blood vessel muscles was produced by stimulating the sympathetic trunk for 15 seconds. The record obtained with the flowmeter is similar to a record of a smooth

muscle contraction obtained with the conventional recording methods. The rapid diminution of flow corresponds to the rising phase of the contraction; the gradual return to the previous level represents the relaxation phase.

General effects of dorsal root stimulation. Immediately after the perfusion is started the blood vessels are completely dilated as shown by the fact that neither dorsal root stimulation nor acetylcholine produces any change of flow. After 1 to 2 hours however the vessels always begin to constrict without any external stimulus. Repetitive stimulation of the dorsal roots then produces, after a latent period of about 3 seconds, a maximal dilatation (fig. 3A). This lasts for about one minute after which the vessels again constrict slowly even though the stimulation of the dorsal roots is continued. After an intermission of a few seconds the

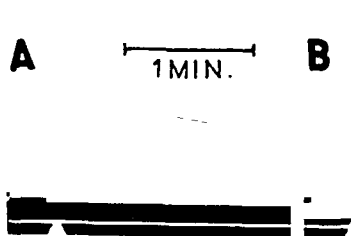


Fig. 2

Fig. 2, A and B. Two contractions of the muscles of blood vessels produced by sympathetic stimulation. During the relaxation of the second contraction, B, the dorsal roots were stimulated. Upper signal marks stimulation of the sympathetic, lower signal that of the dorsal roots.



Fig. 3

Fig. 3, A. and B. Dilatation of the blood vessels of the perfused frog leg, A, on stimulation of the dorsal roots (indicated by the lower of the two signals). B. Application of acetyl- β -methylcholine, 1:2,000,000. The initial constriction of the blood vessels developed spontaneously and was maintained without external stimuli.

stimulation is fully effective again. Another effect of dorsal root stimulation is its influence on the speed of relaxation. Following a strong contraction the relaxation is very slow, but is considerably accelerated by stimulation of the dorsal roots (fig. 2B).

An effect similar to that of dorsal root stimulation can be obtained by injecting acetylcholine into the perfusion fluid (fig. 3B). Following such an injection there is a marked dilatation. Likewise acceleration of the relaxation following strong sympathetic stimulation can be produced by acetylcholine.

Dilatation of blood vessels due to dorsal root stimulation has already been demonstrated in frogs by Pearce (1913) and Doi (1924). Whereas in mammals this reaction occurs mainly in the skin, Krogh, Harrop and Rehberg (1922) showed that in the frog the vessels in skeletal muscles participate to an appreciable extent. My own observations are in agree-

ment with this and further indicate that all the vessels of the perfused leg are affected by the dorsal root fibers. This is shown by the fact that the maximal dilatation produced by dorsal root stimulation is usually as great or nearly as great as that produced by acetylcholine. Removal of the skin left the effect of the dorsal root stimulation practically undiminished in several experiments; in others it was somewhat reduced, due perhaps to the leakage of perfusion fluid through severed blood vessels. Of particular interest in this respect is the observation which will be described in greater detail below, that dorsal root stimulation can completely stop the effect of sympathetic stimulation. This shows clearly that all the vessels which are innervated by constrictor fibers are also supplied by dorsal root fibers.

Effect of dorsal root stimulation on the response to repetitive sympathetic stimulation. The tonic constriction of the blood vessels is, under normal conditions, largely the response to a continuous discharge of impulses transmitted through sympathetic nerve fibers (see Adrian, Bronk and

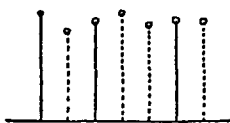


Fig. 4. Influence of dorsal root stimulation on the height of the contraction. Full lines give magnitude of contraction without, interrupted line that with simultaneous dorsal root stimulation. All the contractions produced by stimulating the sympathetic for 5 seconds.

Phillips, 1932). The dilatation produced by the nerves could therefore be explained if it could be demonstrated that the magnitude of the response to sympathetic impulses is reduced by the action of the vasodilators. This question was studied in the following way. A constriction of the blood vessels was produced by stimulating the sympathetic for 5 seconds at a frequency of 20 stimuli per second and the degree of contraction compared with that resulting from a second stimulation during which the dorsal roots were also stimulated. (Actually the dorsal root stimulation was started several seconds before that of the sympathetic on account of the longer latent period of its action.) The result of one of these experiments is given in figure 4 where the height of the contraction is represented by vertical lines. Solid lines are used for the responses to sympathetic stimulation alone and interrupted lines for the response during simultaneous dorsal root stimulation. It is seen that the latter has no measurable influence on the magnitude of the contraction.

Dorsal root stimulation also failed to have any effect on an existing maximal constriction. If the sympathetic was stimulated repetitively and

sufficient time was allowed to produce a constant and maximal contraction, dorsal root stimulation did not cause any dilatation.

Effect of dorsal root stimulation on the response to a small number of

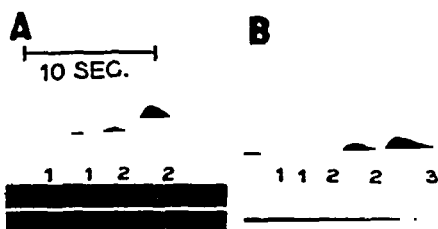


Fig. 5

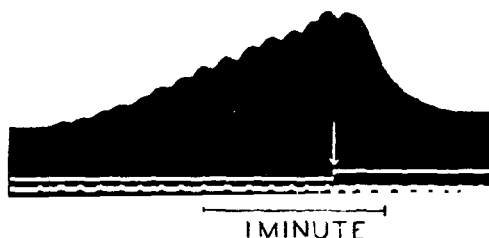


Fig. 7

Fig. 5. Contractions produced by small number of sympathetic volleys; their number is indicated below each contraction. Lower signal marks dorsal root stimulation. Note that a single volley is ineffective during dorsal root stimulation.

Fig. 7. Response of blood vessels to series of sympathetic and dorsal root volleys. One stimulus applied to sympathetic about every 1.5 seconds (marked by lower signal). The arrow indicates the beginning of dorsal root stimulation (20 per sec.) (marked by upper signal).

stimuli. The failure of the dorsal root impulses to diminish the response to sympathetic stimulation seemed at first surprising. The reason for

this negative result was finally found in the fact that the excitatory effect of the sympathetic impulses, at the high frequency used, was so strong that it completely overpowered the inhibitory action of the dorsal roots. This is shown in the following experiments in which the sympathetic was stimulated by only one or a few shocks.

Fig. 6, A. and B. Height of contraction (expressed in millimeter deflection of the flowmeter) as a function of the number of stimuli applied to the sympathetic in a burst (within 1 sec.). Circles represent contractions without, crosses represent contractions during stimulation of dorsal roots (frequency 20 per sec.). The plain circles indicate the first series, the crossed circles the last of the 3 series of observations.

As the number of stimuli in a single burst is increased the influence of the dorsal root stimulation becomes relatively smaller.

This relationship can be expressed quantitatively if the number of stimuli

which are applied to the sympathetic in a given burst is plotted against the height of the contraction produced. In figure 6 the circles represent the contractions produced by sympathetic stimulation alone, the crosses, those produced during simultaneous stimulation of the dorsal roots. The points of the two series fall approximately on two straight lines which are parallel within the limits of accuracy. The inhibitory stimulation has the effect of shifting the line representing the relation between the number of stimuli and the magnitude of the response in the direction of a greater number of stimuli. It makes the first and sometimes the second stimulus of a burst ineffective, but on further increasing the number of shocks the response increases as rapidly as without the inhibitory impulses. If a very large number of stimuli are used one would expect the difference in the response to be very small; this explains the results cited above which failed to show any influence of the stimulation of the dorsal roots.

Although the inhibitory impulses transmitted by the dorsal roots are not able to prevent the contractions produced by a high frequency of sympathetic stimulation it is probable that the frequency of sympathetic discharge in the intact animal is usually sufficiently low for its effect to be controlled by the dorsal root impulses. In fact Bronk, Ferguson, Margaria and Solandt (1936) have recently recorded the impulses in single sympathetic fibers and found them to be of much lower frequency than those in somatic nerves. That such impulse frequencies can nevertheless produce maximal contractions by summation of the individual slow responses is shown by figure 7 where the frequency of stimulation was less than one per second. Such contractions are completely inhibited by dorsal root stimulation (beginning at the arrow) as shown in that same figure.

DISCUSSION. From the observations just reported it may be concluded that the dilator impulses produce their effect by a definite change of the excitability of the muscles of the blood vessels. At a given frequency of dorsal root impulses a certain number of sympathetic impulses are quantitatively inactivated and the muscular activity resulting from the stimulation of the vasomotor nerves appears to be determined by the algebraic summation of the excitatory and inhibitory influences. A simple explanation of the mechanism of this interaction can be given on the basis of the humoral mediation of impulses, and it will be shown that this permits a quantitative explanation of the essential facts which are reported in this paper.

On the basis of present evidence concerning these mediators we assume that stimulation of the dorsal roots produces a dilator substance of the sympathetic fibers, a constrictor substance. We make the further assumption that a certain amount of each of these substances will just "neutralize" each other. It follows then, that the amount of constrictor

substance which is produced by a low frequency of sympathetic impulses will be made just ineffective by the dilator substance produced by the dorsal root stimulation. But if an excess amount of constrictor substance is liberated by additional sympathetic impulses a contraction will result. This describes the relation between the action of the two nerves as found experimentally.

The effect of acetylcholine on the response of the blood vessels is of interest in connection with this theory. High concentrations (1:500,000 of acetyl- β -methylcholine) suppress the response to sympathetic stimulation almost completely. Moderate concentrations (about 1:4,000,000 but very different in different preparations) suppress the response to short periods of stimulation, whereas a maximal response is still obtained by continuous excitation. In the presence of acetylcholine the response to sympathetic stimulation has a considerable latent period (fig. 8).

The theory just outlined implies that the dilator substance has no direct effect on the smooth muscle cells, but merely inactivates a certain amount

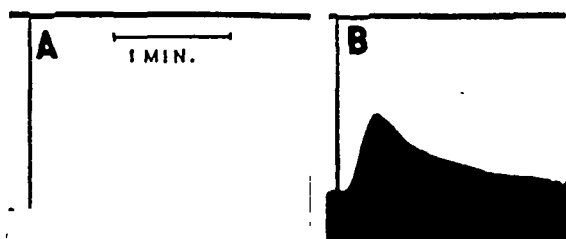


Fig. 8, A. and B. Contractions produced by stimulation of the sympathetic at a frequency of 10 stimuli per second (marked by signal). In B application of acetyl- β -methylcholine 1:4,000,000. Note the longer latent period of the response.

of constrictor substance. The fact that dorsal root stimulation can cause a dilatation in the absence of any sympathetic activity may be explained by the assumption that the tonic contraction of the blood vessel musculature is the result of a process or substance fundamentally the same as that produced by sympathetic impulses. In these respects the inhibition of smooth muscle is apparently different from the vagal action on the heart. The chronotropic effects of both cardiac nerves are exerted directly on the pacemaker. According to Rosenblueth and Simeone (1934) the percentage change of heart rate produced by each of the cardiac nerves is constant and independent of the action of the antagonistic nerve. The effects of the vasoconstrictor and vasodilator impulses on the other hand resemble more closely the c.e.s. and c.i.s. according to Sherrington's conception of the nature of reflex activity (see Sherrington, 1925, and Creed, Denny-Brown, Eccles, Liddell and Sherrington, 1932), because c.i.s. inactivates quantitatively the c.e.s. without acting on the motoneurone directly.

These observations give a simple explanation of the difference between iterative and non-iterative systems, a difference which has been stressed by Lapicque (1926) in order to explain heterochronism in the autonomic effector systems. Thus it had been assumed until recently that a succession of autonomic nerve impulses is necessary in order to elicit a response. However, non-iterative responses have been observed by Gilson (1931), Bishop and Heinbecker (1932) and by Rosenblueth (1932) and we now see that the sympathetic-constrictor system is likewise non-iterative under certain conditions. It does however become iterative under the influence of dorsal root stimulation although it might be argued that in such a case the response to single stimuli is present, but too small to be detected. This however seems improbable when we consider the relationship between the number of stimuli and the strength of the response, given above as a straight line which does not approach or go through the origin (fig. 6A).

The response is also iterative under certain experimental conditions such as at the beginning of the experiment, shortly after the start of perfusion (fig. 6B). The basis for this condition of the muscles which requires repetitive stimulation to elicit a response is probably fundamentally the same as that resulting from the action of the dorsal root impulses, namely the presence of a vasodilator substance. That this is the case is shown by the fact that the perfusate from such a preparation will produce dilatation. The perfusion fluid from a Trendelenburg preparation was collected and injected into another preparation in which the vessels were constricted; a dilatation was produced. This is in agreement with the general conceptions regarding the importance of metabolites in regulating the tone of blood vessels during and after muscular exercise. Thus Rigler (1932) has shown that the perfusion fluid collected from a Trendelenburg preparation after a strong contraction of the skeletal muscles has vasodilator effects. The action of the perfusion fluid during rest is much weaker and probably could not be detected by the usual methods.

The author is greatly indebted to Dr. D. W. Bronk and Dr. H. K. Hartline for valuable criticism.

SUMMARY

1. The vascular responses of a perfused hind leg of the frog have been observed by recording changes in the rate of flow of the perfusion fluid. A sensitive flowmeter suitable for this purpose is described.

2. The question whether vasodilator impulses diminish the response to vasoconstrictor impulses has been studied. It was found that repetitive stimulation of the dorsal roots does not measurably change the response to repetitive stimulation of the sympathetic if the frequency of the sympathetic volleys is high (20 per sec.). The constrictor effect of sympathetic

volleys at a lower frequency (0.5–1 per sec.), however, can be completely suppressed by simultaneous repetitive stimulation of the dorsal roots. As the frequency of impulses in sympathetic nerves under normal conditions is probably small, this mechanism is sufficient to explain the effect of the dilator nerves in the intact animal.

3. Different numbers of stimuli were applied to the sympathetic in rapid succession (within 1 sec.). The response increases linearly with the number of stimuli in a given burst. If the dorsal roots are stimulated simultaneously, a similar relationship is found, but the line representing the variation of the response with the number of stimuli is shifted in the direction of a larger number of stimuli. Whereas usually a single sympathetic volley produces a measurable response, the stimulation of the dilator nerves makes the system iterative.

4. The relation between the effects of the antagonistic nerves can be explained on the assumption that the action of the vasoconstrictor and vasodilator impulses is mediated by neurohormones which inactivate each other and that, consequently, the contraction produced by the stimulation of the vasomotor nerves is determined by the excess of the vasoconstrictor substance.

REFERENCES

- ADRIAN, E. D., D. W. BRONK AND G. PHILLIPS. *J. Physiol.* 74: 115, 1932.
 BISHOP, G. H. AND P. HEINBECKER. *This Journal* 100: 519, 1932.
 BRONK, D. W., L. K. FERGUSON, R. MARGARIA AND D. Y. SOLANDT. *This Journal*, 117, 237, 1936.
 BRÜCKE, E. T. In *Bethe's Handbch. d. norm. path. Physiol.* 10: 29, 1927.
 CREED, R. S., D. DENNY-BROWN, J. C. ECCLES, E. G. T. LIDDELL AND C. R. SHERRINGTON. *Reflex activity of the spinal cord.* Oxford, 1932.
 DOI, Y. *J. Physiol.* 54: 227, 1920.
 GILSON, A. S. *Proc. Soc. Exper. Biol. and Med.* 28: 879, 1931.
 KROGH, A., G. A. HARROP AND P. P. REHBERG. *J. Physiol.* 56: 179, 1922.
 LANGLEY, G. N. AND L. A. ORBELI. *J. Physiol.* 41: 450, 1910.
 LAPICQUE, L. *L'excitabilité en fonction du temps.* 1926.
 PEARCE, R. G. *Ztschr. f. Biol.* 62: 125, 1913.
 RIGLER, R. *Arch. exper. Pharmacol.* 167: 54, 1932.
 ROSENBLUETH, A. *This Journal* 102: 12, 1932.
 ROSENBLUETH, A. AND F. A. SIMEONE. *This Journal* 110: 42, 1934.
 SHERRINGTON, C. S. *Proc. Roy. Soc. B.* 97: 519, 1925.

EFFECTS OF ANATOMICAL SEPARATION OF THE HYPOPHYSIS FROM THE HYPOTHALAMUS IN THE DOG¹

ALLEN D. KELLER, WILLIAM NOBLE AND J. WILLIAM HAMILTON, JR.
From the Department of Physiology and Pharmacology, University of Alabama School of Medicine

Received for publication June 20, 1936

Mahoney and Sheehan (1) recently reviewed in detail the literature dealing with separation or occlusion of the pituitary stalk in the dog. In all such attempts, the physiological effects were those of complete or partial hypophysectomy. Histological studies of the hypophysis substantiated the physiological result. It is clear that investigators lay the blame for the hypophyseal atrophy, which followed such attempts, to an interference in the blood supply. The ease with which the vascular supply to the anterior lobe can be disturbed by stalk separation is readily appreciated from Dandy's (2) and Basir's (3) descriptions of the vascular system of the canine hypophysis. It is generally agreed that there is a communication between the blood channels of the pars anterior and the pars nervosa by way of the sinuses of the pars tuberalis.

METHOD. Dogs in good physical condition, ranging in weight from three to ten kilograms were caged several days previous to the operation. Water was available constantly, and was measured every twenty-four hours, while food remained in the cage from 4:00 p.m. until 8:30 a.m. The food consisted of Balorations² which was mixed with water (60 per cent by weight) at the time of feeding. In records of water consumption, the water in the food was not included. This procedure standardized with considerable exactness the food and water intake over the respective periods. Urine was collected in covered jars and measured every twenty-four hours. Following operation the same regime was maintained until the termination of the experiment. Blood sugar determinations were made just previous to, and immediately after operations, and for several days thereafter. Rectal temperatures were recorded daily for ten days to two weeks following operation.

OPERATIVE PROCEDURE. The hypophysis was approached by the subtemporal route. After the exposure was completed, the stalk was cut

¹ Aided by a grant from the Rockefeller Foundation.

² Balorations is a prepared, well-balanced dog food obtained from Tioga Mills, Inc., Waverly, New York.

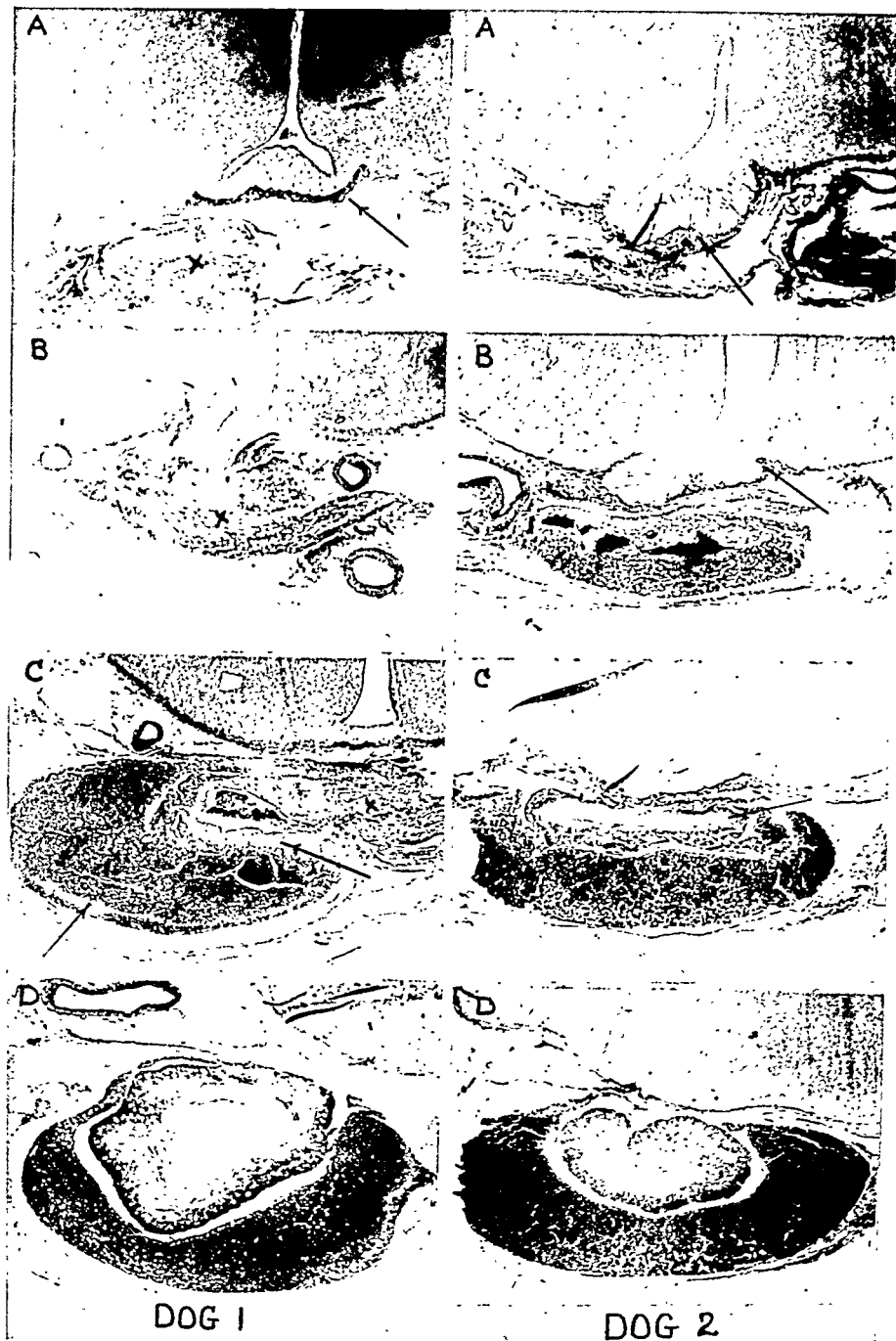


Fig. 1. Photomicrographs of serial sections. Dog 1, terminated 15th day.

Note: A. The strap of pars tuberalis remaining attached to the brain anteriorly, indicated by arrow. Muscle which was inserted between separated gland and hypothalamus, indicated by X. B. Muscle, indicated by X. C. Distal portion of infundibulum, indicated by arrow; intact anterior lobe except for slight area of necrosis, indicated by arrow; pars tuberalis bounding infundibulum, and muscle

by scissors, attempting to make the section such that the whole of the gland, including the infundibulum, was severed from the hypothalamus. The successful accomplishment of this procedure has been described and illustrated elsewhere (4) as the first step in hypophysectomy.

RESULTS. Clean severance of the hypophysis from the hypothalamus was attempted in five dogs. In two of these, a bit of muscle was placed in the line of separation, while in the other three no precaution was taken to prevent approximation of the cut surfaces. For the first few days after operation, the dogs showed a mild increase in water intake, as well as a possible trace of increased food consumption. Following this initial rise water intake fell below normal, in some cases to zero, for a few days, returning subsequently to normal or slightly above normal (see curves on dogs 2 and 3 in fig. 2). There was no significant alteration in blood sugar, rectal temperature, or in the general behavior of the animals, either directly following operation or subsequently.

Serial section of the hypophysis and hypothalamus revealed that in all animals the anterior strap of pars tuberalis remained attached to the brain base (see fig. 1, dog 1-A), and in two cases a bit of the thickened portion of the pars tuberalis remained anteriorly (see fig. 1, dog 2-A). In all instances there was a complete separation of the remainder of the gland including the greater portion of the infundibulum. In three experiments there was no macroscopic degeneration in the pars tuberalis, anterior lobe, pars intermedia, or infundibulum peripheral to the lesion. In one instance there was considerable atrophy in the anterior lobe, while in the remaining case only a trace was present (see fig. 1, dogs 1-C and D). In all cases there was a characteristic central disappearance of tissue in the infundibular process such that there was a direct continuation of the infundibular cavity (distal tip of the third ventricle) in to the infundibular process (see fig. 1, dog 1-D and dog 2-D). An increased cellularity in the tissue of the infundibular process which remained was a constant finding (see fig. 3).

In another group of four animals immediately after the gland had been severed, its proximal surface was cauterized slightly about the region of the severed stalk. This is easily possible in most instances without involvement of the hypothalamus because the gland after separation retracts somewhat into the fossa. Three of these dogs exhibited a much

plug. D. Intact anterior lobe except for small area of necrosis, and central atrophy of the infundibular process.

Dog 2, terminated 36th day.

Note: A. Tip of pars tuberalis intact on brain base anteriorly. B. A small strap of pars tuberalis intact on brain base; distal to section the pars tuberalis essentially undisturbed, and undisturbed anterior lobe. C. Infundibulum, pars tuberalis and anterior lobe all undisturbed. D. Intact pars anterior and pars intermedialis, but characteristic central atrophy of infundibular process.

more marked increase in water consumption for the first few days following operation than did those of the preceding series. The temporary diabetes insipidus in these animals equalled the maximum seen in our series and ranged from 600 to 800 cc. of water per kilogram of body weight per day. After several days the water intake returned to normal or a trace above normal, where it remained until termination of the experiment. The fourth dog of this group exhibited, directly following operation, only a slight increase in water consumption which subsided to zero on the ninth day, then rose rapidly to a high level in the characteristic fashion of the permanent phase of diabetes insipidus as described by the Ranson group

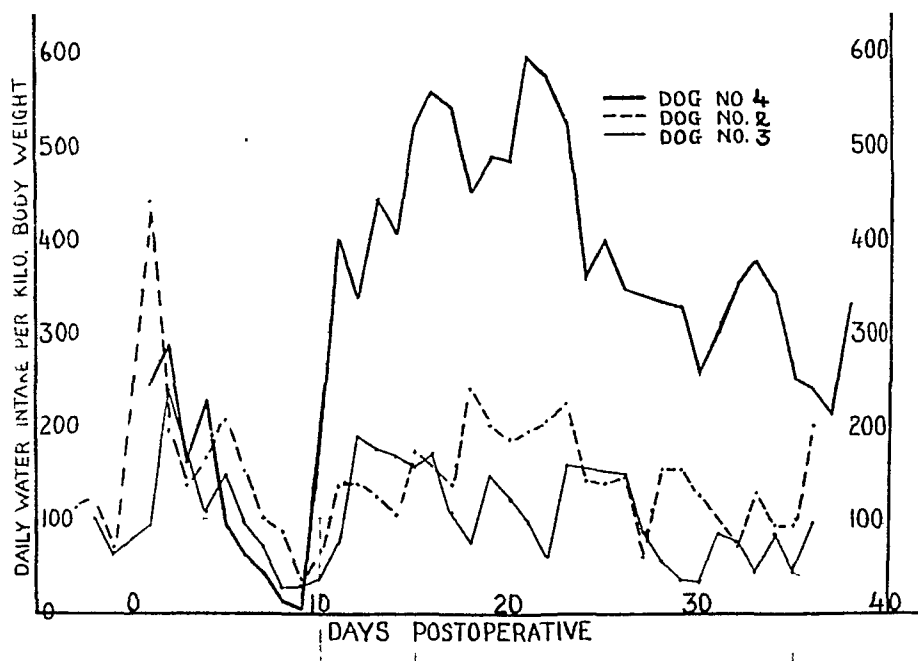


Fig. 2. Dogs 2 and 3 had the stalk cut, without detectable injury to gland as illustrated in figure 1. In dog 4 subsequent to cutting the stalk the gland was cauterized slightly about the area of entrance of the stalk into the gland.

(5) (see fig. 2, curve dog 4). The food intake of this group of animals, though it increased slightly following operation in two animals, remained essentially normal. In these two dogs a definite tendency to adiposity was evident.

Macroscopic study of serial sections of the hypophysis revealed varying degrees of involvement of the pars tuberalis and pars anterior. In all instances, however, considerable tissue of each category remained. In the case of dog 4 there was very little infringement upon the pars tuberalis and none upon the pars anterior. In all experiments there was a hollowing out of the infundibular process with an increased cellularity in the

remaining portion. In no case did the pars intermediate seem encroached upon. The whole of the gland, including the infundibulum, was separated in dogs 5 and 4. Dog 5 exhibited no deviation from the normal subsequent to the tenth day.

DISCUSSION. It is readily apparent from the photographs shown in figure 1 that an adequate blood supply to the anterior lobe of the canine hypophysis remains after a clean separation of the gland from the hypothalamus. Further, the cautery experiments demonstrate that considerable encroachment upon the pars tuberalis is possible without evident disturbance in the vascular supply to the remaining portion of the gland. It is to be stressed that such a surgical result was to be expected from the description of the vascular supply of the gland as given by Dandy and Basir. It is evident that in previous attempts at severance or occlusion

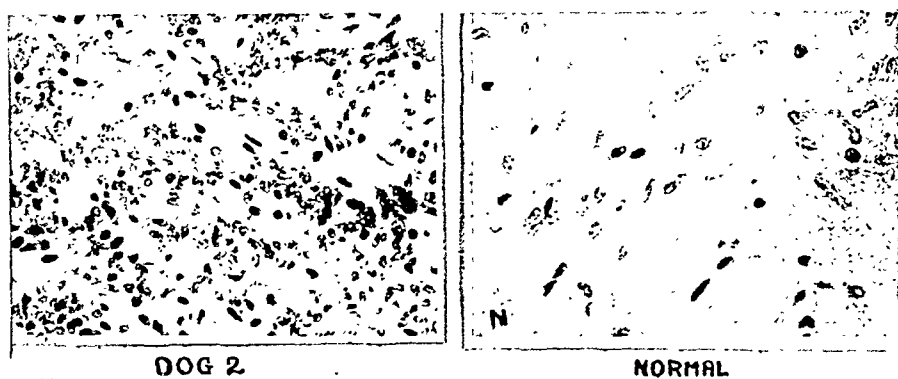


Fig. 3. Right is a photomicrograph taken from the infundibular process of an unoperated dog.

Left is a photomicrograph taken from the infundibular process remaining in dog 2. Both sections were stained with cresyl violet and have same magnification.

of the stalk in the dog the anastomosis through the pars tuberalis has been greatly encroached upon.

The changes which occurred in the infundibular process appear to be equivalent to those described by Fischer, Ingram, Hare and Ranson (4) in the cat following hypothalamic lesions. These investigators attribute the changes to section of the nerve fibers passing to this structure from the anterior hypothalamus. Although we have encountered these results following hypothalamic lesions, such that these fibers would have been cut, we are not certain but that a mechanical factor is in part responsible.

The absence of increased water consumption following clean stalk sections in our experiments demonstrates that denervation of the infundibular process is not the cause of the diabetes insipidus which follows hypothalamic lesions as has been postulated by the Richter and Ranson schools. Furthermore the precipitation of the permanent phase of dia-

betes insipidus by trauma to the proximal portion of the separated gland demonstrates that the essential mechanism necessary for the production of both the temporary and permanent phases of diabetes insipidus is inherent in the hypophysis itself.

We hasten to point out that in the dog, as well as in the cat, diabetes insipidus of varying severity frequently follows bilateral lesions placed with the needle in the hypothalamus. The incidence in the cat is strikingly less than in the dog. The curve on dog 5 in figure 4 illustrates such

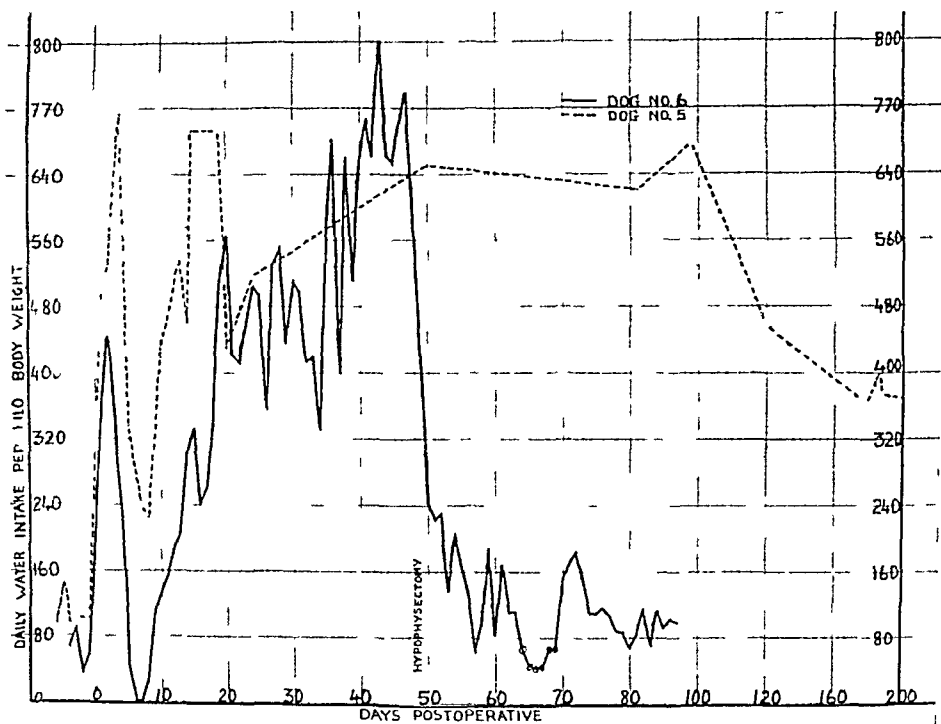


Fig. 4. Both experiments are described in the text. In dog 6, following hypophysectomy, some milk was given in addition to water and the total fluid intake is graphed. The values circled represent water intake on days when milk was not given and are therefore directly comparable with values graphed previous to hypophysectomy.

an experiment. If diabetes insipidus following such hypothalamic lesions is not the result of hypophyseal denervation, what then is the mechanism concerned? In the light of the foregoing results a neighboring involvement of the hypophysis would seem more probable than the possibility of an additional independent neural mechanism located in the hypothalamus.

That the hypophysis is essential for the diabetes insipidus induced by a hypothalamic lesion is evidenced by the curve on dog 6, shown in figure 4. In this dog hypophysectomy, on the 49th day after the central lesion

was placed, eliminated abruptly a striking diabetes insipidus. This experiment does not necessarily demonstrate that the direct cause of the increased water consumption produced by the hypothalamic lesion was due to a disturbance in the gland, because the effect of the hypophysectomy on metabolism might be sufficient to eliminate the diabetes insipidus.

Exactly the same paradox exists relative to enhanced appetite and resultant adiposity. Enhanced food consumption occurs in instances following 1, hypothalamic lesions (6), and 2, removal of the posterior lobe of the hypophysis (7), yet stalk section has been accomplished with no disturbance in food consumption or body weight. Immediately following operation there is a marked increase in the amount of food eaten and for the first three or four weeks there is a progressive rapid rise in body weight. In severe cases food intake is limited by the capacity of the stomach, food being repeatedly regurgitated, then eaten again. Eventually food intake returns to normal and body weight is in turn either maintained or returns slowly to normal. To this degree there occur here also the temporary and permanent phases. It is to be recalled that a tendency to adiposity occurred in two instances where the hypophysis was encroached upon by cautery, also a typical case of enhanced appetite with adiposity has been encountered (8) following gland separation where the hypophysis was encroached upon by pressure with forceps.

SUMMARY

The hypophysis was successfully separated from the hypothalamus 1, without infringement upon the adequacy of the blood supply to the gland, or 2, without inducing enhanced food or water consumption.

Experiments are described and recalled which demonstrate that enhanced food intake and diabetes insipidus can be produced by involvement of the hypophysis alone.

An experiment is described and illustrated wherein a severe diabetes insipidus elicited by a hypothalamic lesion was eliminated abruptly by hypophysectomy.

REFERENCES

- (1) MAHONEY, W. AND O. SHEEHAN. *Brain* **59**: 61, 1936.
- (2) DANDY, W. E. AND E. GOETSCH. *Am. J. Anat.* **11**: 137, 1911.
- (3) BASIR, M. A. *J. Anat.* **66**: 387, 1932.
- (4) KELLER, A. D. AND M. C. D'AMOUR. *Arch. Path.* **21**: 185, 1936.
- (5) FISHER, C., W. R. INGRAM, W. K. HARE AND S. W. RANSON. *Anat. Record* **63**: 29, 1935.
- (6) KELLER, A. D., W. K. HARE AND M. C. D'AMOUR. *Proc. Soc. Exper. Biol. and Med.* **30**: 772, 1933.
- (7) KELLER, A. D. AND W. NOBLE. *This Journal* **113**: 79, 1935.
- (8) KELLER, A. D. AND W. NOBLE. *Proc. Amer. Physiol. Soc.*, 1936.

THE MEASUREMENT OF SERUM VOLUME

F. WILLIAM SUNDERMAN AND J. H. AUSTIN

From the John Herr Musser Department of Research Medicine and the Pepper Laboratory of Clinical Medicine, University of Pennsylvania

Received for publication June 25, 1936

In estimating the quantity of any component present in blood serum, the measurement of serum volume is obviously essential. The methods for this measurement, however, have not given entirely satisfactory and reproducible results. The results with different methods and with the same method by different investigators have conspicuously shown lack of agreement. Of the various methods devised, the dye method has been the one most generally employed. In this method a solution of a non-toxic dye, such as brilliant vital red, is injected intravenously into a subject and from its dilution in the serum, the volume of the serum is estimated.

The method originally proposed by Keith, Rowntree, and Geraghty (1) and later modified by Smith, Whipple and associates (2) has been discussed by Erlanger (3), Lamson and Rosenthal (4), Peters and Van Slyke (5) and others. It has been pointed out that in the measurement of serum volume by the dye method the main discrepancies are concerned first, with respect to the accuracy of measuring the dye which is introduced; second, the accuracy with which the blood sample obtained after injection represents a uniform mixing; third, the extent to which the dye remains in the circulation during the time taken for mixing; and fourth, the accuracy of the colorimetric measurement. The studies which are here reported are directed chiefly toward the consideration of these problems.

Colorimetry of vital red. Gregerson, Gibson and Stead (6) have indicated that one of the chief problems in the measurement of serum volume by means of the dye method concerns itself with the colorimetry of vital red. Inasmuch as vital red dissolved in normal saline and added to serum yields a different color value than when it is dissolved in water and added to serum, studies were made to determine the nature of the color produced by vital red in solutions of different ionic concentrations, of different ion species and in the presence of serum.

Apparent differences of concentration of 0.004 per cent vital red in water and salt solution at various pH values with Wratten no. 74 and no. 75 filters.

In table 1 is shown the composition of various solutions of dye in water, phosphate buffer, NaCl, KCl and NaI solution. The solutions all containing the same concentration of dye were compared in a colorimeter using in the lens system either a no. 74 Wratten filter with transmission as shown in figure 1, maximal at $\lambda 530 \text{ m}\mu$ and falling to 10 per cent of maximal at $\lambda 510$ and $560 \text{ m}\mu$, or a no. 75 Wratten filter with transmission maximal at $\lambda 485 \text{ m}\mu$ and falling to 10 per cent of maximal at $\lambda 465$ and $515 \text{ m}\mu$. A is the solution of dye in water and its apparent concentration is taken as unity for comparison with the other solutions. B is the dye in phosphate buffer, pH = 8.0; D and E, in NaCl solution; C, F and H, in NaCl plus buffer; G, in NaI plus buffer; I, J and K in KCl plus buffer;

TABLE 1

Influence of salts on the colorimetric reading of 0.004 per cent vital red in water

DESIGNATION	pH	IONIC STRENGTH	$\frac{\text{Na}}{\text{M}}$	$\frac{\text{K}}{\text{M}}$	$\frac{\text{Na} + \text{K}}{\text{M}}$	$\frac{\text{Cl}}{\text{M}}$	$\frac{\text{I}}{\text{M}}$	$\frac{\text{H}_2\text{PO}_4}{\text{M}}$	$\frac{\text{HPO}_4}{\text{M}}$	APPARENT FILTER #74	CONCENTRATION FILTER #75
A	?	μ								1.00	1.00
B	8.0	0.029	0.019	0.0005	0.020			0.0005	0.010	0.94	
C†	8.0	0.150	0.109	0.002	0.111	0.030		0.002	0.039	0.83	
D	?	0.150	0.150		0.150	0.150				0.86	
E	?	0.300	0.300		0.300	0.300					0.77
F*	8.0	0.400	0.359	0.002	0.361	0.280		0.002	0.039	0.79	
G	8.0	0.400	0.359	0.002	0.361		0.280	0.002	0.039	0.69	
H	5.9	0.150	0.050	0.090	0.140	0.030		0.090	0.010	0.74	
I	5.9	0.379	0.079	0.290	0.369	0.259		0.090	0.010	0.60	
J	8.0	0.389	0.079	0.271	0.350	0.269		0.002	0.039	0.61	
K†	8.0	0.400	0.079	0.282	0.361	0.280		0.002	0.039	0.58	
L	?	0.300		0.300	0.300	0.300					0.62

* Average reading of two solutions of identical composition.

† Average reading of three solutions of identical composition.

and L, in KCl. Comparison of A, B, C and F with no. 74 filter shows decreasing color intensity as Na^+ increases. Comparison of C and D indicates that Cl decreases the color less than HPO_4^- . Comparison of F and G indicates that Cl decreases the color less than I^- . Comparison of E and L and of F and K indicates that, with either filter Na^+ decreases the color less than K^+ . Comparison of H and I, with no. 74 filter, indicates decreasing color with increasing K^+ . Comparison of I and J indicates that if Na^+ and K^+ are kept approximately constant, change in pH has little effect on color intensity.

Apparent concentration of dye added to serum with or without added salt with Wratten no. 74 and no. 75 filters. In table 2 is shown a series of additions of dye to 0.004 per cent concentration in 75 per cent dog serum

in water (M) or in NaCl or KCl solution (N to R). With both filters the color of the dye added to serum, without added salt (M) is taken as unity. For expressing ionic strength and Na^+ and K^+ concentrations the serum was assumed to contain 140 mM/L Na^+ , 5 mM/L K^+ and $\mu = 150$. N, O and P were prepared with added KCl; Q and R, with added NaCl. With a no. 74 filter both KCl and NaCl increased the apparent concentration of the dye instead of diminishing it, as in the absence of serum. In the presence of serum Na^+ was more effective than K^+ in producing this change. With a no. 75 filter the changes are very slight, within the error of measurement.

TABLE 2
Influence of salts on colorimetric reading of vital red in serum

DESIGNATION	IONIC STRENGTH	$\frac{\text{Na}}{\text{M}}$	$\frac{\text{K}}{\text{M}}$	$\frac{\text{Na} + \text{K}}{\text{M}}$	APPARENT CONCENTRATION	
					Filter #74	Filter #75
0.004 per cent dye in 75 per cent dog serum	μ					
M	0.113	0.105	0.004	0.109	1.00	1.00
N	0.123	0.105	0.014	0.119	1.00	1.00
O	0.213	0.105	0.104	0.209	1.05	1.00
P	0.313	0.105	0.204	0.309	1.14	1.01
Q	0.213	0.205	0.004	0.209	1.14	1.01
R	0.313	0.305	0.004	0.309	1.19	1.01
0.0066 per cent dye in 75 per cent human serum						
S	0.113	0.105	0.004	0.109		1.00
T	0.277	0.269	0.004	0.273		0.97

With 75 per cent human serum containing 0.0066 per cent dye the addition of NaCl slightly reduced the apparent concentration with a no. 75 filter.

The change of color of vital red with time in aqueous solution (0.002 to 0.005 per cent) was tested with both a no. 74 and no. 75 Wratten filter, reading against a neutral tint filter with an optical density of 1.0. The maximum variation with the no. 74 filter was 0.2 mm. and with the no. 75, 0.1 mm. in a total reading of about 10 mm. over a period of $1\frac{1}{2}$ hours. A consistent weakening of color (0.1 to 0.3 mm.) was observed after 18 hours.

Spectrophotometry of vital red solution in water and in 75 per cent dog's serum with or without added NaCl or KCl. All solutions represented in figure 1 were prepared to contain 0.0040 per cent or 0.0024 per cent dye in the following solvents: W, water; Na W, 0.3 M NaCl solution; K W,

0.3 M KCl solution; S, 75 per cent dog serum diluted with water; NaS, 75 per cent dog serum with aqueous solution of NaCl added to 0.2 M in addition to the salt of the serum. C S represents the reading of 75 per cent dog serum without added dye. The solutions were read in a 1 cm. cell with a Bausch and Lomb spectrophotometer of the König-Martens type with the technique of Drabkin and Austin (7). In reading W, Na W and K W the compensating cell contained water. In reading S and Na S the compensating cell contained 75 per cent serum without dye; and the total absorption would accordingly be the sum of each of these curves with the curve C S in figure 1. The extinction coefficient is calculated

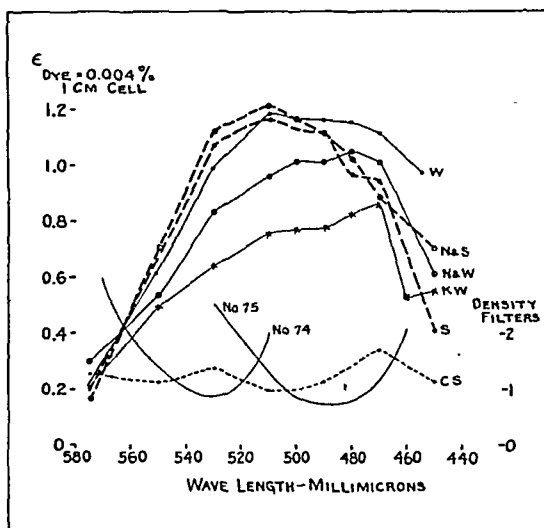


Fig. 1. The influence of salts and serum on spectral absorption of vital red (0.004 per cent solution). W, dye in water; NaW, dye in 0.3 M NaCl; KW, dye in 0.3 M KCl; S, dye in 75 per cent dog's serum diluted with water; NaS, dye in 75 per cent dog's serum in aqueous NaCl to give final concentration of 0.2 M NaCl in addition to the salt of the serum; CS, 75 per cent dog's serum diluted with water, no dye. Curves 74 and 75 show the optical density of the Wratten filters employed.

for 0.004 per cent vital red in 1 cm. cell. The same graph shows the optical density of the no. 74 and no. 75 Wratten filters used with the colorimeter. The curves show the depressed absorption of the dye present in the NaCl solution to that of water, and the still greater effect on adding a normal KCl solution. They show displacement of the curve toward the red on adding serum and slight increase of absorption on adding NaCl to the serum. These observations are consistent with the colorimetric observations reported, and the greater consistency of readings with the no. 75 filter as compared with the no. 74 could be predicted.

These studies indicate the importance of the type of standard originally recommended by Keith, Rowntree and Geraghty, namely, one in which

the vital red is dissolved in the serum of the patient to be tested. The difficulty arising from a ratio of dye to serum in the standard differing from that in the unknown, hence preventing perfect matching, can be readily obviated if the apparent color is a linear function of concentration of dye added to serum over a range that includes all of the unknowns. If two standards be prepared by adding dye to the serum obtained before

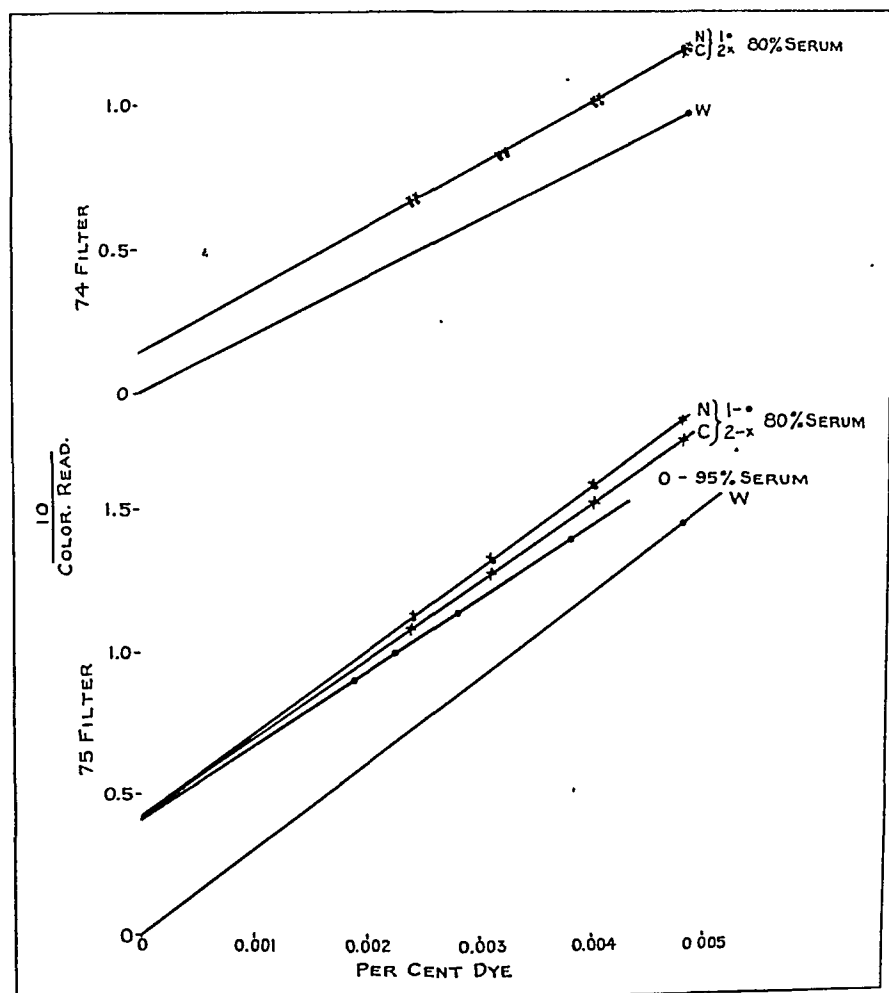


Fig. 2. Linear increment in light absorption as vital red is added to serum. *N*, *C* and *O*, dye added to human serum (see text). *W*, dye added to water.

injection of dye so that one standard is weaker than the weakest unknown and the other standard stronger than the strongest unknown, the reciprocals of the colorimeter readings obtained with a neutral tint standard and an appropriate color filter might furnish a line on which might be interpolated the reciprocals of the readings of any of the unknowns to determine the concentration of dye present.

Figure 2 shows a test of this on five human sera, two bleedings an hour apart on subject *N*, two bleedings similarly on subject *C* and one bleeding on subject *O*. With the first four sera to 2 cc. portions was added 0.5 cc. of varying concentrations of vital red in water to give final concentrations shown on the abscissae. Each of the 16 resulting mixtures was read with both the no. 74 and no. 75 Wratten filter. With subject *O*, to 2 cc. portions of serum was added 0.1 cc. of varying concentration of dye in water. In addition a reading with each filter was taken of 0.0048 per cent dye in water, *W*. The final concentration of serum for *N* and *C* was 80 per cent; for *O*, 95 per cent. The absorption of the diluted sera without dye was read with the no. 75 filter but was too weak to read with the no. 74 filter. It is evident that the relations are satisfactorily linear. With the no. 75 filter the slope in serum is a trifle less steep than in water.

TABLE 3

Influence of hemoglobin on colorimetric reading of vital red (No. 75 Wratten filter)

SERUM MIXTURE*	CONCENTRATION OF HEMOGLOBIN	COLORIMETRIC READINGS #75 FILTER	POSITIVE BENZIDINE REACTION
	<i>mgm. per 100 cc.</i>	<i>mm.</i>	<i>dilution</i>
1	0.0	5.4	1 to 10 with NSS
2	10.5	5.4	1 to 50 with NSS
3	21.0	5.1	
4	42.0	5.1	
5	84.0	4.7	

* The serum mixtures were prepared by adding to 4 cc. of serum, 0.5 cc. vital red in aqueous solution and 0.5 cc. of hemoglobin solutions of various concentrations prepared from hemolyzed cells. All mixtures contained 0.0048 per cent final concentration of vital red.

Thus far little superiority is indicated for one of the two color filters over the other. However, a matter of great importance for this colorimetry is interference arising from traces of oxyhemoglobin contaminating the serum. As one of the two bands of this pigment is at $\lambda 540 \text{ m}\mu$ with rapid falling off of the absorption toward $\lambda 490 \text{ m}\mu$ it can be predicted that the no. 75 filter will give results less sensitive to disturbance from traces of hemoglobin. A trace of hemoglobin so small that bands are barely visible with a small spectroscope increased the apparent concentration of the vital red using the no. 74 filter by 33 per cent, whereas with the no. 75 filter the increase was only 7 per cent. It is evident, however, that even using the latter filter, precautions must be taken to exclude disturbing contamination of the sera with traces of hemoglobin.

Vital red measurement in serum containing hemoglobin. Experiments were undertaken to determine the critical concentration of hemoglobin

that might be present in serum containing vital red and yet still be blocked out with the use of the no. 75 Wratten filter. For the colorimetric readings a neutral gray glass with an optical density of 1.0 was employed as the standard. The results of these analyses are given in table 3. It will be seen that hemoglobin present in serum containing vital red up to at least a concentration of 10.5 mgm. per 100 ml. had no influence upon the colorimetric readings. Employing the benzidine test according to the procedure of Lyle, Curtman and Marshall (8), a positive reaction was obtained when serum was diluted 1 to 10 with normal salt solution and

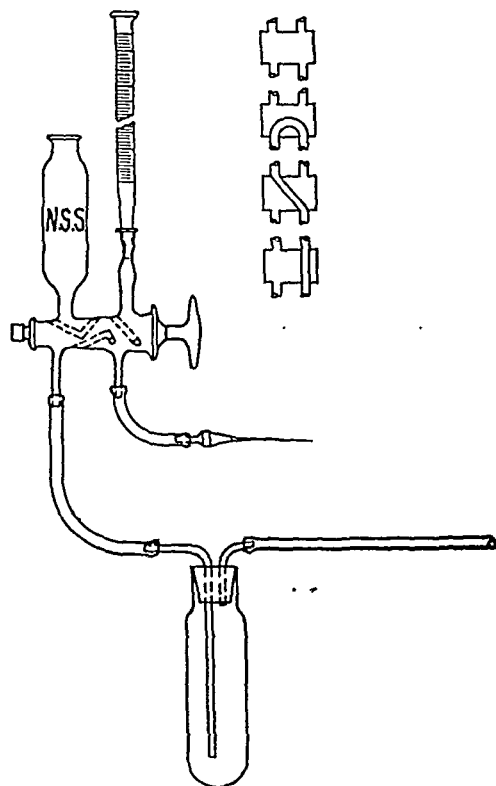


Fig. 3. Apparatus for the intravenous injection of vital red

a barely detectable reaction was obtained in a 1 to 25 dilution. In the serum to which hemoglobin was added to give an added concentration of 10.5 mgm. of hemoglobin per 100 ml., a positive benzidine reaction was obtained in a 1 to 50 dilution and a barely detectable reaction was obtained in a 1 to 200 dilution with normal salt solution. Thus it was possible to measure vital red in serum containing added hemoglobin when that serum gave a positive benzidine reaction in a dilution 5 times greater than was necessary to give the same reaction in serum not containing added hemoglobin.

With the method of double centrifugation of serum described below

positive benzidine reactions are obtained only in dilutions not exceeding 10- to 25-fold. Any specimen of serum should certainly be excluded for the purpose of this technique if it reacts positively to benzidine in 5 times this dilution. In our experience the various specimens of sera from the same individual have been closely comparable in their benzidine reaction.

Method for measurement of serum volume. The modifications we have devised for the estimation of serum volume permit of precise measurement of the dye introduced into the vein; allows adequate time for homogeneous mixing; takes into account the rate of disappearance of the dye from the serum; and permits of an accurate colorimetry of the dye present in the serum regardless of species.

The measurement is made under basal conditions after the subject has rested in bed for 30 minutes before the introduction of the vital red. The dye is introduced by means of the apparatus shown in figure 3. This apparatus consists of a four-way glass stopcock, a reservoir for normal salt solution, a burette for the dye, and a removable centrifuge tube for the collection of the initial sample of blood. The needle is placed into the vein and the blood is collected into the centrifuge tube by gentle suction. The blood remaining in the rubber tubing between the needle and the stopcock is washed back into the vein with the normal salt solution. The dye, accurately measured from a small burette graduated in 1/50 cc., is then introduced. It has been found convenient to introduce 1 cc. of a 2 per cent solution of vital red per 10 kgm. of body weight. The dye remaining in the tubing is finally washed completely into the circulation with normal salt solution.

At intervals of 30, 60 and 90 minutes after the introduction of the dye, 5 or 6 cc. samples of blood are removed without stasis from the opposite arm. The blood is allowed to stand at room temperature for at least 15 minutes, then the clot is *gently* separated from the sides of the centrifuge tube by means of a glass rod. The serum is removed after the clotted blood has been centrifuged, and the serum is again *re-centrifuged* in order to separate any erythrocytes which occasionally are carried over from the first separation. The concentration of dye present in each sample of serum is then determined.

To 2 cc. amounts of the serum obtained before the introduction of dye are added 0.5 cc. amounts respectively of 0.01 and 0.02 per cent of an aqueous solution of vital red. These solutions are then read directly in a micro colorimeter employing a neutral gray standard with an optical density of approximately 1.0 in place of the colorimeter cup and a double thickness no. 75 Wratten green filter in the lens system. An incandescent 100 Watt Tungsol headlight bulb in a Bausch and Lomb attachable illuminator is employed as the source of light. A plot of the reciprocal of the colorimeter readings against the concentrations of dye present is

made, and a straight line through the points is used to estimate the concentration of dye from the colorimetric readings of given samples taken after injection. To 2 cc. amounts of the sera obtained after the introduction of dye, 0.5 cc. of water is added. The readings of the dye present in the samples are made using the same neutral gray standard. The concentration of dye present in each sample is then obtained by interpolating the reciprocals of the colorimeter reading on the plot of the known concentrations of dye added to the original sample of the same patient's serum.

The concentration of dye in the samples of serum obtained at intervals of 30, 60 and 90 minutes gives in our experience a linear relationship. If we assume that this justifies linear extrapolation back to the moment of injection we can estimate the concentration of dye that would have been

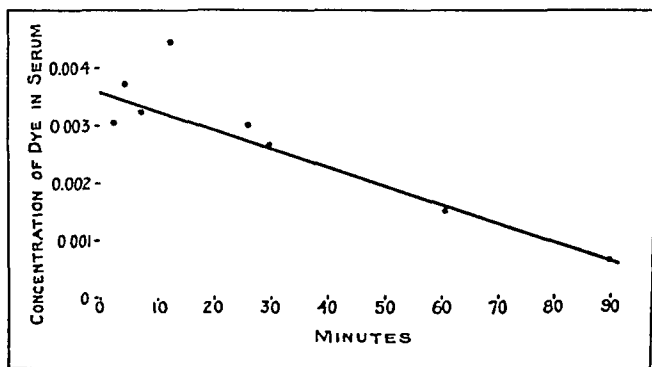


Fig. 4. Concentrations of vital red in peripheral blood serum from 2 to 90 minutes after injection. For explanation see text.

obtained at this moment were complete mixing instantaneous. Having obtained this value the serum volume may then be calculated.¹

Selection of time intervals. In preliminary studies it was noted that the simultaneous removal of blood from both arms within the first twenty minutes after the introduction of the dye did not give the same concentrations of dye in the two samples, whereas after about twenty minutes, the same concentrations were obtained. In figure 4 is given an example of the concentrations of dye obtained at varying time intervals after the injection of dye into a dog. The scattering obtained during the first twenty minutes is demonstrated.

It might be assumed that the rate of disappearance of dye would be directly related to its concentration and that the disappearance would

¹ In order to correct for inadequate mixing and the disappearance of dye from the circulation, Erlanger in 1921 suggested that it might be possible to determine the concentration of dye at the time of injection by means of a backward extrapolation from the latter parts of the curve of disappearance.

be more rapid at first, following perhaps a semilogarithmic curve. The concentrations of dye at 30, 60 and 90 minute intervals when plotted semilogarithmically have given practically the same extrapolated values as when plotted arithmetically, the change in concentration over the period studied being too small to distinguish which type of curve is followed.

The serum volume in normal and pathological conditions. In figure 5 are given nine measurements of the serum volume in 5 normal male students between the ages of 20 and 25 years. The four duplicate determi-

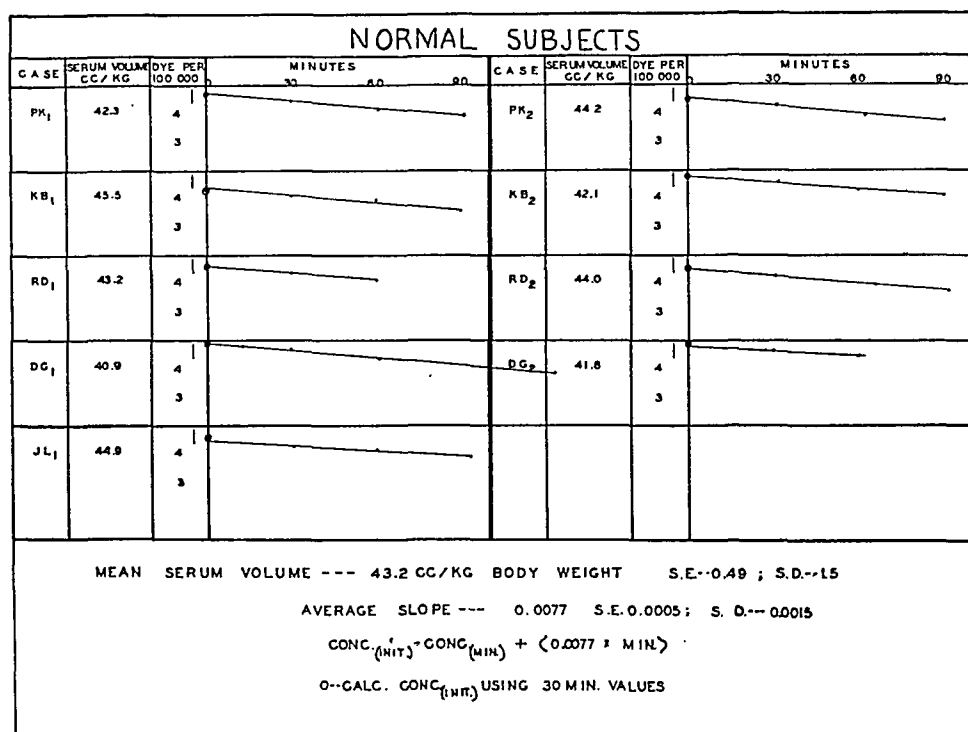


Fig. 5. The measurement of serum volume in normal individuals. For explanation see text.

nations were made at intervals of a week or more. The observed concentrations are shown at 30, 60 and 90 minutes and the estimated initial concentration in parts per 100,000 by the line extrapolated to 0 minute. The vertical lines represent the observed range of these nine normals obtained when 1 cc. of the 2 per cent dye per 10 kgm. of body weight was injected. In the second column are given the values for the serum volume, calculated as cubic centimeters of serum per kilogram of body weight. The mean of these nine determinations is 43.2 cc. with S.D. of 1.5 cc. indicating as the normal range about 40 to 46 cc. per kilogram of body weight.

The average slope of the concentration curves is 0.0077 cc./kilo body weight/minute with S.D. of 0.0015. The constancy of this slope in normal individuals is sufficient to permit calculation of the initial concentration of dye in the serum from the concentration in a single sample obtained at 30 minutes after injection by the use of the following equation:

$$\text{CONC. (initial)} = \text{CONC. (min.)} + (0.0077)(\text{min.})$$

The initial concentrations calculated from this equation and from the 30 minute values are represented by the open circles at 0 minutes.

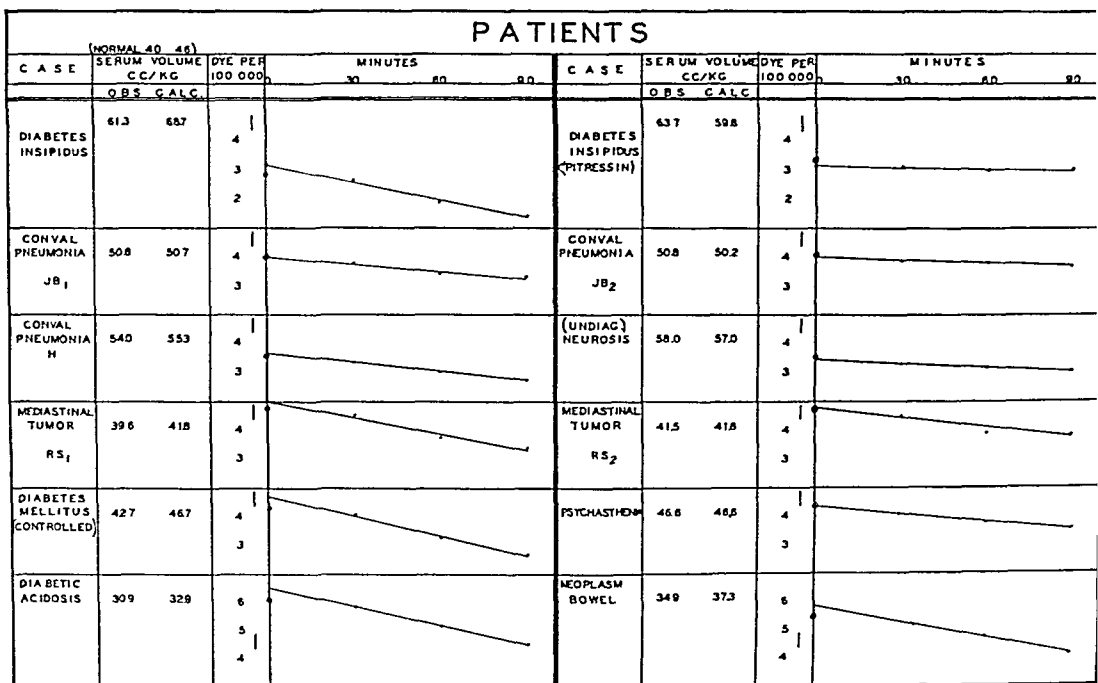


Fig. 6. The measurement of serum volume in some miscellaneous pathological conditions. For symbols see text relating to figure 5.

In figure 6 are given the measurements of the serum volume in a few patients suffering with various pathological conditions. This graph serves to demonstrate the scattering in the values which may occur pathologically. High values were obtained in one patient with diabetes insipidus and in three convalescing from pneumonia; low values have been obtained in one patient with diabetic acidosis and in one with malignancy of the bowel. The values indicated on the graph by the extrapolated line have been obtained by the three point method; the calculated values indicated by the open circle at 0 minute have been obtained by the equation using only the 30 minute values. It will be seen that the ob-

served and calculated values show sufficient agreement to render the one point method of value when repeated puncture is undesirable.

Since our measurements have all been made on serum, the question arises whether the concentration of dye in the serum is changed in the process of clotting. Hooper and his associates (9) have already demonstrated that vital red remains completely in the plasma and is not taken up by the erythrocytes. The determination of the concentration of dye in serum from a sample of blood which had been allowed to clot and the calculation of the concentration of dye in the same sample of blood to which isotonic $\text{Na}_2\text{C}_2\text{O}_4$ had been added, showed no significant difference.

It should be pointed out that there is no method available for determining whether the results with this procedure represent the absolute serum volume; indeed, the definition of absolute serum volume would be open to debate. The method, however, gives consistent reproducible results in normal individuals and reveals difference beyond the normal range in some pathological cases and thus should be of practical value.

Our sincere thanks are expressed to Dr. Norman P. Shumway who assisted one of us (FWS) in some of the preliminary studies.

SUMMARY

The studies demonstrate a marked change in the color values of vital red dissolved in aqueous solutions of salts of different ionic strength and ion species, with altered effects in the presence of serum.

A technique of colorimetry has been developed in which two standards are prepared by adding dye to serum obtained from the subject before injection of dye, one standard weaker than the weakest unknown, and the other standard stronger than the strongest unknown. The standards are compared in a colorimeter against a neutral tint filter having an optical density of 1.0 and with a double thickness no. 75 Wratten filter in the lens system. A straight line drawn through the reciprocals of these readings furnishes a valid basis for interpolation of reciprocals of the unknowns similarly read, and from this may be determined the concentration of dye in these unknowns.

A method of using the benzidine reaction has been developed for the purpose of detecting the presence of hemoglobin in serum containing vital red.

In the determination of the serum volume by the intravenous injection of vital red, there is evidence to suggest that complete mixing of the dye in the circulating serum does not occur for twenty to thirty minutes after the injection. If samples of serum are obtained under basal conditions at intervals of 30, 60 and 90 minutes after the introduction of the dye, a linear relationship is obtained which permits extrapolation to the

moment of injection and this furnishes we believe the best available estimate of the concentration of dye that would have been obtained were complete mixing instantaneous.

The quantity of dye injected is measured with precision by means of an especially constructed apparatus.

REFERENCES

- (1) KEITH, N. M., L. G. ROWNTREE AND J. T. GERAGHTY. *Arch. Int. Med.* **16**: 547, 1915.
- (2) SMITH, H. P., H. R. ARNOLD AND G. H. WHIPPLE. *This Journal* **56**: 336, 1921.
- (3) ERLANGER, J. *Physiol. Reviews* **1**: 177, 1921.
- (4) LAMSON, P. D. AND S. M. ROSENTHAL. *This Journal* **63**: 358, 1923.
- (5) PETERS, J. P. AND D. D. VAN SLYKE. *Quantitative clinical chemistry*, Vol. II, Methods. The Williams & Wilkins Co., 701, 1932.
- (6) GREGERSEN, M. I., J. J. GIBSON AND E. A. STEAD. *Proc. Am. Physiol. Soc.*, p. 54, 1935.
- (7) DRABKIN, D. L. AND J. H. AUSTIN. *J. Biol. Chem.* **98**: 719, 1932.
- (8) LYLE, W. G., L. J. CURTMAN AND J. T. W. MARSHALL. *J. Biol. Chem.* **19**: 445, 1914.
- (9) HOOPER, C. W., H. P. SMITH, A. E. BELT AND G. H. WHIPPLE. *This Journal* **51**: 205, 1920.

THE EFFECT OF LACTATION AND EXERCISE ON THE RATE OF INVOLUTION OF THE UTERUS IN THE RAT

ELIZABETH ABBOTT AND A. C. IVY

*From the Department of Physiology and Pharmacology, Northwestern University
Medical School, Chicago, Illinois*

Received for publication July 6, 1936

Although obstetricians generally believe that nursing promotes the involution of the uterus in the human mother (1-12), we have been unable to find satisfactory data proving this belief. In the rat Kurmitsu and Loeb (13) measured the thickness of sections of the uterine wall at various intervals after parturition in non-lactating, lactating, castrated and lactating, and castrated and non-lactating animals. They concluded that lactation and castration hasten involution, and that the effect of lactation is exerted directly on the uterus and not indirectly through the ovaries. A report of the effect of exercise on involution of the uterus has not been found. This work was undertaken to determine the effect of lactation and exercise on the rate of involution of the uterus by actually weighing the uterus sixteen days after delivery in lactating rats, non-lactating-resting rats, and non-lactating-exercised rats.

METHODS. Albino rats from the same colony and over 100 days old were used. None of the rats had been pregnant prior to these experiments. They were fed the Steenbock diet ad libitum with the addition every two or three days of fresh carrots and beef liver. Litter mates were divided between either two of three groups, namely, a lactating group, a non-lactating group, and a non-lactating-exercise group in about one-half of the instances, or in so far as was practically possible. The rats in the lactating group were permitted to nurse all their pups. The pups were removed from the non-lactating group as soon after parturition as possible. The pups were removed from the non-lactating-exercise group and their mothers subjected to 20 minutes of swimming (water, 20°C.) in two ten minute periods daily, care being taken to dry them carefully after the swimming exercise. The exercise periods were not longer than 10 minutes because we did not care to exhaust the animals; even ten minutes tired them considerably. Floating was prohibited, and we believe the exercise was more standard than could be obtained by a tread-mill method.

The rats were killed 16 days after parturition and during the period of dioestrus. In the absence of lactation, oestrus appeared early (13, 14)

and in some instances on the sixteenth day, which necessitated the discarding of some animals. The stage of oestrus was determined by the vaginal smear method and further checked by examining the lumen of the uterus. Only animals in dioestrus have been admitted to our data. Rats of various weights were employed to ascertain any difference that might arise from the weight of the animal at the onset of its first pregnancy.

The uterus was removed by sectioning at the tubes and at the vagina near the cervix. It was then fixed for one day in a ten per cent neutral solution of formalin in 0.9 per cent sodium chloride. Then the extraneous tissue was dissected away; the uterus was carefully dried with filter paper and weighed to within 5 mgm. The body weight of the rat was determined at intervals prior to and after mating, and after death by ether.

We have arranged the data shown in the tables in order of the body weights at autopsy, rather than in the order of the weight at mating because the correlation between the uterine:body-weight ratios in the various body-weight groups was closer when the data were arranged according to the final body weight. This might appear to be obvious, but it had to be examined because of the variable effect of pregnancy and lactation, or both, on the body weight of the rats (15).

Our exercise periods were determined in the following manner: The time of involution of the uterus of a nursing rat is seven days (that for a non-nursing rat from 21 to 28 days), after which significant changes do not occur. The average time for the uterus of a nursing human mother is usually given as six weeks. The ratio between the two (7:42) is one-sixth. Hence, we thought that ten minutes of exercise in the rat should be equivalent, in regard to rate of uterine involution, to sixty minutes in the human. Since we should not consider it practical to exercise a human mother (after the period of hospitalization) fairly vigorously more than one-hour daily in one-half hour periods separated by a rest period, we exercised a group of rats (10) for ten minutes—two five-minute exercise periods—which had no effect on the rate of uterine involution. We then increased the exercise to two ten-minute periods, obtaining the results reported later. Twenty minutes of exercise in the rat would be equivalent to two hours in the human.

RESULTS. *The effect of lactation.* The data showing the effect of lactation on the rate of involution of the uterus are given in table 1. It matters not how the data are grouped or analyzed, lactation is found to markedly augment the rate of involution. At 16 days post-partum the average weight of the uterus of the lactating rat is approximately 58 per cent less than the average weight of the uterus of the non-lactating-resting control rats.

It is to be noted that the non-lactating rats during pregnancy and the

TABLE 1

NON-LACTATING (25), RESTING RATS					LACTATING (39) RATS					NON-LACTATING, EXERCISED (31) RATS					
Rat number	Litter number	Body weight before pregnancy	Body weight at 16 days	Weight of uterus	Rat number	Litter number	Body weight before pregnancy	Body weight at 16 days	Weight of uterus	Rat number	Litter number	Body weight before pregnancy	Body weight after delivery	Body weight at 16 days	Weight of uterus
		gm.	gm.	mgm.			gm.	gm.	mgm.			gm.	gm.	gm.	mgm.
57	11	190	224	250	5	10	220	220	85	23a	3	180	200	210	225
59	8	200	222	270	7	11	220	220	100	29b	13	190	—	204	210
66	7	200	214	270	4a	—	172	212	110	20c	10	190	160	200	220
61	5	198	214	345	4	10	210	210	90	28b	11	176	—	198	210
52	—	190	214	285	5a	3	150	210	105	24c	11	176	164	194	275
50	12	200	218	330	8	6	218	210	100	4b	7	160	200	194	255
6	8	200	212	220	6a	8	200	210	100	28c	7	162	190	190	235
31	8	190	210	200	2a	8	170	208	105	12c	9	150	172	188	250
43	3	180	210	280	3a	—	177	204	105	25a	7	154	180	186	150
11	8	200	209	160	3	10	200	200	95	3b	11	180	198	186	230
60	6	200	200	220	22	8	192	200	95	15b	12	172	184	186	225
Group A ↑					29	8	190	200	160	24a	8	152	124	184	175
Ave. A.	7.6	195	213	257	53	6	192	200	115	1b	13	146	184	184	210
10	11	185	192	305	Ave. A.	8.0	193	208	105	19c	10	180	160	180	205
13	7	174	190	215	55	11	194	194	110	35a	10	130	138	180	165
9	10	170	184	150	68	6	190	192	95	21c	7	170	186	180	205
20	5	180	180	205	2	12	190	190	115	Group A ↑					
27c	11	178	192	245	33	9	212	190	100						
Group B ↑					13b	8	210	190	120						
					16b	10	168	190	100						
					65	7	210	186	110						
					54	12	198	180	125						
					3b	11	202	180	130						
					10b	12	210	180	80						
Ave. B.	8.8	177	187	224	Ave. B.	9.8	197	187	108						
Ave. AB.	8.0	190	205	247	Ave. AB.	8.8	195	199	106	Ave. A.	9.3	167	174	190	215

TABLE 1—*Concluded*

NON-LACTATING (25), RESTING RATS					LACTATING (39) RATS					NON-LACTATING, EXERCISED (31) RATS					
Rat number	Litter number	Body weight before pregnancy	Body weight at 16 days	Weight of uterus	Rat number	Litter number	Body weight before pregnancy	Body weight at 16 days	Weight of uterus	Rat number	Litter number	Body weight before pregnancy	Body weight after delivery	Body weight at 10 days	Weight of uterus
		gm.	gm.	mgm.			gm.	gm.	mgm.			gm.	gm.	gm.	mgm.
14a	11	160	176	300	45	8	194	178	80	5c	8	140	142	178	235
17a	14	160	176	230	12b	9	188	174	115	2b	12	140	188	178	205
18c	9	170	172	260	13a	12	160	174	100	4c	11	140	160	174	265
7a	—	172	170	240	47	5	170	170	100	42a	11	136	162	172	150
16a	6	140	170	245	20b	2	174	170	125	20a	—	130	173	170	160
19b	7	156	162	220	23b	5	182	162	100	30a	7	132	174	170	235
16	8	160	160	190	18b	4	176	160	80	11c	10	135	160	170	220
18a	11	140	160	220	17b	8	150	160	95	22c	7	156	158	170	160
10a	9	165	160	215	46	9	188	160	85	46a	5	130	146	168	185
Group C ↑					12a	10	144	160	95	27a	9	154	170	166	200
					Ave. C.	7.2	173	167	97	29c	11	170	164	164	190
Ave. C.	8.1	158	167	235	34a	8	170	154	75	40a	5	136	156	164	200
Group D					9a	4	130	150	100	3c	9	150	168	160	175
					22b	7	180	150	75	23c	9	168	158	160	200
					8a	10	120	146	105	26c	4	166	160	160	165
					11b	8	180	144	75	Group B ↑					
					6b	8	160	140	135						
					Ave. D.	7.5	156	147	94	Ave. B.	8.4	145	162	168	196
					Ave. CD.	7.3	167	160	96						
Ave. all	8.4	178	191	243	Ave. all	8.2	183	183	102	Ave. all	8.9	156	168	180	206

The non-lactating rats gained an average of 7.8 per cent, approximately, in body weight during pregnancy and lactation. The lactating rats gained an average of 3.4 per cent during pregnancy and lost this gain during lactation. The exercise group gained an average of 9 per cent, approximately, during pregnancy and 15 per cent during pregnancy and lactation.

post-partum period gained 7.8 per cent in body weight, while the group of rats that nursed young showed no gain. However, the lactating rats during pregnancy made an average gain of 3.4 per cent, which gain was lost as a result of lactation, the loss and gain in weight ranging between 9 and 15 per cent respectively. This variation in body weight incident to lactation caused us to average the weights of the uteri of the lactating rats that either lost, gained or showed no change in body weight during lactation. The results were: the average weight of the uterus of the rats that gained body weight during the lactation period was 101 mgm.; of the rats that lost, 101 mgm.; of the rats showing no change, 100 mgm. This showed that the change in body weight during the lactation period had no appreciable effect on the rate of involution of the uterus. The average weight of the uteri of the lactating rats that gained during both pregnancy and lactation was 105 mgm.; of those that lost, 100 mgm.; of those that showed no change, 101 mgm. Although we doubt that this difference is significant, the trend is in the direction that might be anticipated over a growth period of thirty-seven days (pregnancy plus lactation) (15), and coincides with our calculations that the uterine body weight ratios of all the rats in the different body weight groups correlates better when the rats are arranged on the basis of the final rather than the initial body weight.

No satisfactory correlation could be made between litter size and the rate of involution of the uterus. This argues against nitrogen demand as an important factor in the rate of involution.

None of the lactating rats manifested an oestrous cycle prior to the 16 day period. This effect of lactation is well known (13, 14). All of the non-lactating rats manifested an oestrous cycle prior to 16 days and exercise did not appreciably delay the post-partum onset of oestrus (average time of onset of oestrus in non-lactating-resting group, 6.9 days; in the non-lactating-exercise group, 6.6 days).

The effect of exercise. The effect of the two ten-minute daily periods of rather vigorous exercise on the rate of involution of the non-lactating rat is not nearly as definite as the effect of lactation. The data on 31 rats are given in table 1. It is to be noted that the exercised group gained considerable body weight (average 9 per cent) during pregnancy, and continued to gain (average, 6 per cent additional) during the post-partum exercise period. In fact, this group gained more than the non-lactating-resting group. This may have been due to the smaller rats in the exercise group; but it is quite evident that the exercise did not affect the post-partum weight as did lactation.

Because the difference between the mean weight of the uteri of the non-lactating-resting rats and that of the non-lactating-exercised rats was relatively small in contrast to the lactating rats, the probable error of the

differences between the means had to be determined. The essential data are given in table 2. In group I (table 2) the difference between the mean of all the non-lactating-resting rats and the mean of 16 non-lactating-exercised rats selected so as to yield a similar final weight is 28 (± 8.3), which is a significant difference. The difference between the means, in group II, the method of comparison being reversed, is 22 (± 7.82), which is also significant, but not quite so definite. In group III the data are compared on the basis of the initial rather than the final body weights. As might be expected the difference, 18 (± 9.24), is not so

TABLE 2

GROUP		LITTER AVER- AGE NUM- BER	AVER- AGE INITIAL BODY WEIGHT	AVER- AGE FINAL BODY WEIGHT	AVER- AGE UTER- INE WEIGHT	DIFFERENCE WITH P.E. OF DIFFERENCE IN THE MEANS
			grams	grams	mgm.	
I	All of non-lactating, resting rats (25)	8.4	178	191	243	28 (± 8.3)
	16 non-lactating, exercised rats: Group A, table 1	9.3	167	190	215	11.5%
II	18 non-lactating, resting rats, including rats 31, 43, 11, 60 in group A, table 1, and rats in groups B and C	8.3	170	181	228	22 (± 7.82) 10.6%
	All of non-lactating, exercised (31) rats, table 1, groups A and B	8.9	156	180	206	
III	A selected group of non-lactating, resting rats (13), selected so as to yield an initial body weight of 171 gm. Initial body weight 185-156 gm. Final body weight 212-160 gm.	8.7	171	180	231	18 (± 9.24) 7.7%
	A selected group of non-lactating, exercised rats (15), selected so as to yield an initial body weight of 171 gm. Initial body weight 190-156 gm. Final body weight 204-164 gm.	9.0	171	187	213	

significant; yet, the difference again shows that exercise had some effect in increasing the rate of involution of the uterus. Expressed in percentage, we believe that data show that the exercise increased the rate of involution 11 per cent, which is considerably less than the 58 per cent increase caused by lactation.

Our observations on the effect of pregnancy and lactation on the body weight of the rat in general compare favorably to those of Simmonds (16), whose data show that the average body weight remains stationary (range about -15 to +50 per cent; our range, -9 to +15 per cent).

DISCUSSION. Our observations on the effect of lactation on the rate

of involution of the uterus confirm by a different method those of Kuramitsu and Loeb (13). Our method, we believe, permits a more satisfactory quantitation.

Three factors should be thought of in considering the effect of lactation on the rate of involution of the uterus: One, a nitrogen demand resulting from lactation; two, the mammo-uterine reflex which causes contraction of the uterus, and hence improvement in blood supply and lymph drainage and in turn facilitates the natural tendency of the post-partum uterus to involute; and third, endocrine factors. In regard to the first factor, our evidence indicates that on an *ad libitum* diet the factor of nitrogen demand is not a chief factor. This is indicated by the failure of the decrease in uterine weight to correlate satisfactorily in the lactating rats whether they gained, lost or manifested no change in body weight during the lactation period. The relatively slight effect of exercise may have been due to such a factor; in fact that is one reason why we were interested in determining the effect of exercise and were surprised in regard to our findings. Yet, the effect of exercise may have been due to stimulation of circulation and possibly to some reflex stimulation of uterine activity. In regard to this factor the effect of thyroid feeding and a sub-maintenance, low protein diet should be studied. We believe the second factor, the mammo-uterine reflex, to be very important. Such a reflex has been definitely demonstrated in monkeys by Ivy, Hartman and Koff (17). The endocrine factor is undoubtedly very important. Lactation delays the onset of the ovarian-uterine cycle as revealed by the vaginal changes. The ovarian-uterine cycle was not appreciably delayed by the exercise, which in addition to the absence of the mammo-uterine reflex explains why exercise exerted relatively little effect on the rate of involution. That the effect of the ovaries is marked is shown by the results of Kuramitsu and Loeb (13). The effect of castration after parturition, however, is not as great as the effect of lactation, since nursing still exerts a slight effect on involution after castration. In fact, Kuramitsu and Loeb conclude that probably the changes resulting in the uterus after castration render difficult the realization of the effects of either the lack of nursing or of nursing. Since oestrin increases the irritability of the uterine musculature (and promotes vascularity), a uterus under the influence of some oestrin would be more responsive to the mammo-uterine reflex effects.

SUMMARY AND CONCLUSIONS

1. When the uteri of non-lactating (25) and lactating rats (39), receiving a standard diet *ad libitum*, were weighed sixteen days after parturition, it was found that the mean weight of the latter group was 58 per cent less than that of the former group. Thus, lactation increases the rate of involution of the uterus very decidedly.

2. When non-lactating rats (31) were exercised daily for sixteen days after parturition, the mean weight of the uteri was decreased only by 11 per cent as compared with non-lactating-resting controls. Thus, moderately vigorous exercise only slightly augments the rate of involution of the uterus.

REFERENCES

- (1) POLAK. *Am. J. Obst. and Gynec.* **13**: 437, 1927.
- (2) GOODALL. *Am. J. Obst. and Gynec.* **60**: 921, 1909.
- (3) TEACHER. *J. Obst. and Gynec. of the British Empire* **34**: 11, 1927.
- (4) MARSHALL. *Physiology of reproduction*. 2nd ed., London, 1922.
- (5) WEBSTER. *Research in female pelvic anatomy*. Part 1, p. 5.
- (6) WILLIAMS. *Am. J. Obst. and Gynec.* **22**: 683, 1931.
- (7) LONGRIDGE. *Brit. Med. J.* **2**: 1230, 1910.
- (8) SLEMONS. *Johns Hopkins Hosp. Bull.* **25**: 198, 1914.
- (9) ENGELTHORN. *Arch. f. Gynak.* **96**: 1, 1912.
- (10) FROMMEL. *Ztschr. f. Geburts. u. Gynak.* **7**: 305, 1889.
- (11) THORN. *Ztschr. f. Geburts. u. Gynak.* **16**: 57, 1889.
- (12) VINEBERG. *Am. Gynec.* **1**: 125, 1902.
- (13) LOEB AND KURAMITSU. *Am. J. Physiol.* **55**: 423, 443, 1921; **56**: 40, 1921.
- (14) LONG AND EVANS. *The oestrous cycle in the rat and its associated phenomena*. Berkeley, 1922.
- (15) DONALDSON. *The rat*. 2nd ed., Philadelphia, 1924.
- (16) SIMMONDS. *Am. J. Hyg.* **4**: 1, 1924 (Supp.).
- (17) IVY, HARTMAN AND KOFF. *Am. J. Obst. and Gynec.* **22**: 396, 1931.

THE CROSSED RESPIRATORY IMPULSES TO THE PHRENIC

A. ROSENBLUETH AND T. ORTIZ¹

From the Department of Physiology in the Harvard Medical School

Received for publication July 7, 1936

Langendorff (1887) and Girard (1890) observed contractions of one half of the diaphragm in rabbits and dogs after an ipsilateral semisection of the spinal cord above C₃ and severance of the contralateral phrenic nerve. Schiff (1894) and Porter (1895) showed that the crossing of the respiratory impulses did not occur until the phrenic was cut on the opposite side. Porter further demonstrated that this crossing occurred at the level of the phrenic nuclei, not above or below.

We have failed to find any reports of further work on this phenomenon since Porter's observations. All the explanations which have been offered to account for it seem to contradict some known properties of nerve impulses or to postulate new, unparalleled properties of the nerve cells. Thus, Schiff (1894) spoke of the section of a phrenic acting as a "specific enhancing agent" of the activity of the opposite phrenic. He laid great stress on asphyxia, but did not mention the possible blocking of afferent impulses by the section. Porter (1895) suggested that some dendrites of the phrenic motoneurons cross over; the impulses they carry are not usually sufficient to cause a contraction of the opposite side of the diaphragm; but, after section of the phrenic nerve a greater portion, perhaps the whole of the "descending impulse" of that side, passes through the crossed dendrites into the phrenic cells of the opposite side. Similarly, Barcroft (1934) speaks of the impulse coming down the side where the phrenic is cut being "thwarted" along its usual path, therefore "pushing" across to the opposite phrenic along which it discharges.

The present study was undertaken with the purpose of obtaining, if possible, a satisfactory explanation of the "crossed phrenic phenomenon." We felt that before new properties are postulated for nerve cells or synapses all the possibilities of explaining the data in terms of the known properties should be exhausted. The experiments quoted did not control for the possible rôle of afferent nerve impulses and asphyxia.

METHOD. With dial (Ciba) anesthesia at the doses (per kgm.) bracketed, the following animals were used: monkeys (0.5 cc.), dogs (0.7 cc.),

¹ Mexican Fellow of the Guggenheim Foundation.

cats (0.75 cc.), guinea pigs (0.5 cc.), rabbits (0.55 cc.) and woodchucks (0.6 cc.). The dial was injected intraperitoneally and supplemented with ether or urethane whenever necessary.

The vagi and phrenics were approached in the neck. For stimulation of the cut nerves, buried shielded electrodes were employed, delivering shocks from a Harvard induction coil with 5 volts in the primary circuit. Reversible blocks of the phrenics were obtained by applying pledgets of cotton soaked with ether to the dissected nerves at the base of the neck until paralysis of the corresponding hemidiaphragm was complete. The sections of the spinal cord and dorsal roots were all performed acutely. These sections were verified macroscopically at autopsy.

A tracheal cannula was inserted for artificial respiration, whenever

TABLE 1

	MON-KEYS	DOGS	CATS	GUINEA PIGS	RAB-BITS	WOOD-CHUCKS
Respiratory hemiplegia on spinal semi-section.....	Yes	Yes	Some-times	Yes	Yes	Yes
Crossed diaphragmatic contractions on cutting the vagi, cervical sympathetics and depressors.....	No	Yes	No	No	No	No
Crossed diaphragmatic contractions with asphyxia (see text for exceptions)....	No	No	No	No	No	?
Crossed diaphragmatic contractions on cutting the active phrenic.....	No	Yes	Yes	No	Yes	Yes
Crossed costal movements.....	No	No	No	No	No	No
Unilateral costal respiration adequate...	Yes	Yes	Yes	No	No	?
Unilateral diaphragmatic respiration adequate.....	Yes	No	Yes	Yes	Yes	Yes

necessary, and to produce asphyxia, either by closing the cannula or by connecting it to a rubber balloon containing nitrogen or expired air.

The movements of both halves of the diaphragm were recorded as follows. A midline abdominal incision was made from the umbilicus to the base of the xiphoid cartilage. Two long clamps were placed on the abdominal walls, immediately below the ribs. Sometimes a third clamp was fixed to the xiphoid cartilage. The clamps were lifted by a horizontal rod so that the diaphragmatic region of the animals was slightly raised from the animal board. This procedure was found not to interfere with respiration. After the abdominal viscera were pressed caudad with cotton, which also served to protect them from unnecessary exposure, the anterior half or more of the diaphragm was accessible and visible. Serrefines were then placed symmetrically toward the center of each dome

and connected, via pulleys, to the recording levers. Downward excursions of these denote, therefore, inspiration.

This procedure was adopted for recording because it was deemed of importance to be able to observe the diaphragm directly, in order to distinguish clearly active from passive movements. Other respiratory movements such as the costal, abdominal, laryngeal and nasal, were also observed directly.

RESULTS. *A. Monkeys.* A spider monkey and three macaques were studied. In three of them a semisection of the spinal cord at C₂ totally paralyzed all the thoracic respiratory movements on the same side; the

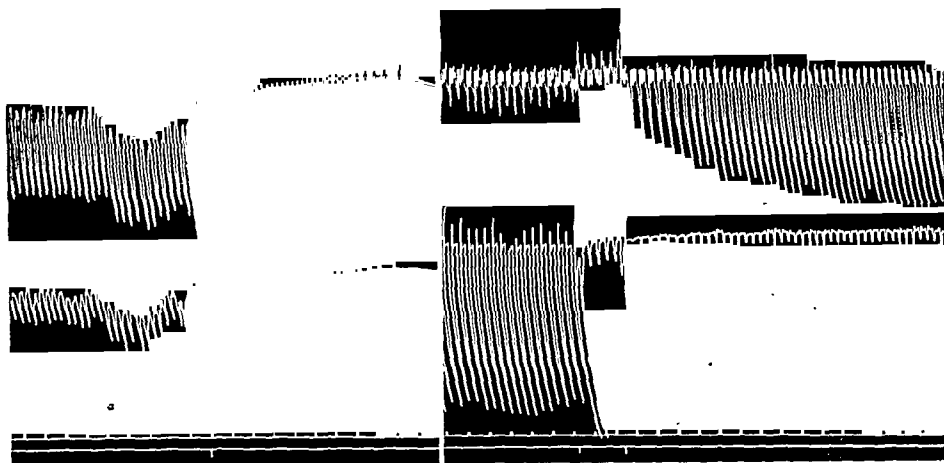


Fig. 1

Fig. 2

Fig. 1. Macaque monkey. Left spinal semisection at C₂; complete transection at C₇. Vago-sympathetics cut. Upper record: right, and lower record: left half of the diaphragm. At lower signal, section of the right phrenic. The movements occurring after this section were transmitted from the neck and head muscles. In this and the succeeding figures the upper signal records 5 second intervals.

Fig. 2. Dog. Left spinal semisection at C₂; complete transection at C₇. Upper record: left, and lower record: right half of the diaphragm. Between the lower signals, section of the right phrenic.

fourth monkey will be described separately. In one of the macaques a complete transection of the cord was also made at C₇, eliminating all costal respiration. Cutting the vago-sympathetic trunks (two animals) induced only slight typical effects on the active, but no change on the paralyzed side. Section of the discharging phrenic failed in the three animals to elicit respiratory movements of the semisected side. The animal which had had the complete transection at C₇ died of asphyxia, after the larynx and nose had shown the movements which correspond to labored breathing and to gasps (fig. 1). The other two monkeys survived the section of the phrenic, the costal breathing of the active side sufficing

to maintain an adequate ventilation; they were sacrificed 40 and 60 minutes later, respectively, without having shown any further diaphragmatic contraction.

In the fourth monkey, a macaque, a left spinal semisection at C_3 failed to paralyze the ipsilateral diaphragmatic contractions, although they were smaller than those of the other hemidiaphragm. An ether block (see p. 502) was applied to the right phrenic, whereupon the entire diaphragm was paralyzed. On recovery from this block the left phrenic was cut, but the contractions of the left hemidiaphragm, which had now reappeared, were not abolished by this section. Cutting the right phrenic permanently paralyzed all the diaphragm. Obviously the contractions of the left hemidiaphragm occurred in response to nerve impulses delivered by the right phrenic, a case of peripheral crossing. With the possible

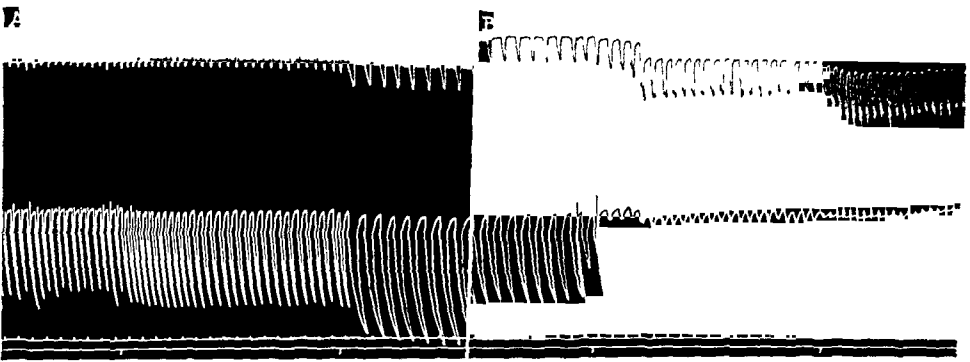


Fig. 3. Dog. Right spinal semisection at C_2 ; complete transection at C_7 . Upper record: right, and lower record: left half of the diaphragm.

A. Section of the left, and then the right vago-sympathetic nerves.

B. Section of a branch, then of the remainder of the left phrenic.

exception of some cats (see section C), this was the only unquestionable instance of a peripheral crossing of the phrenic nerve supply encountered in all the animals studied. Since the ether block resulted in total paralysis of the diaphragm we may conclude that in this monkey, as in the other three, the crossed phrenic phenomenon did not occur.

B. *Dogs*. A spinal semisection at C_2 and a complete transection at C_7 were performed on 6 dogs; in 5 others only the semisection at C_2 was made. In all cases the diaphragm and the costal muscles were paralyzed on the side of the semisection. Cutting the phrenic on the opposite side, with the vagi intact, promptly resulted in respiratory movements of the previously paralyzed half of the diaphragm in one animal out of 3 (fig. 2). The two other dogs also belonged to the group which had a spinal transection at C_7 . Cutting the active phrenic in these two animals led to immediate asphyxia; artificial respiration and severance of the vagi suc-

ceeded in bringing about adequate diaphragmatic contractions in the opposite half of the diaphragm in only one of them.

In the remaining 8 dogs the vago-sympathetic nerves were cut before section of the phrenic. With one exception, these cuts immediately led to the sharing of the previously paralyzed hemidiaphragm in the respiratory movements (fig. 3A). Subsequent section of the originally active phrenic increased only slightly the contractions of the opposite side (fig. 3B). The exception mentioned was a dog with only a spinal semisection at C₂ in which cutting the vagi did not lead to respiration of the paralyzed

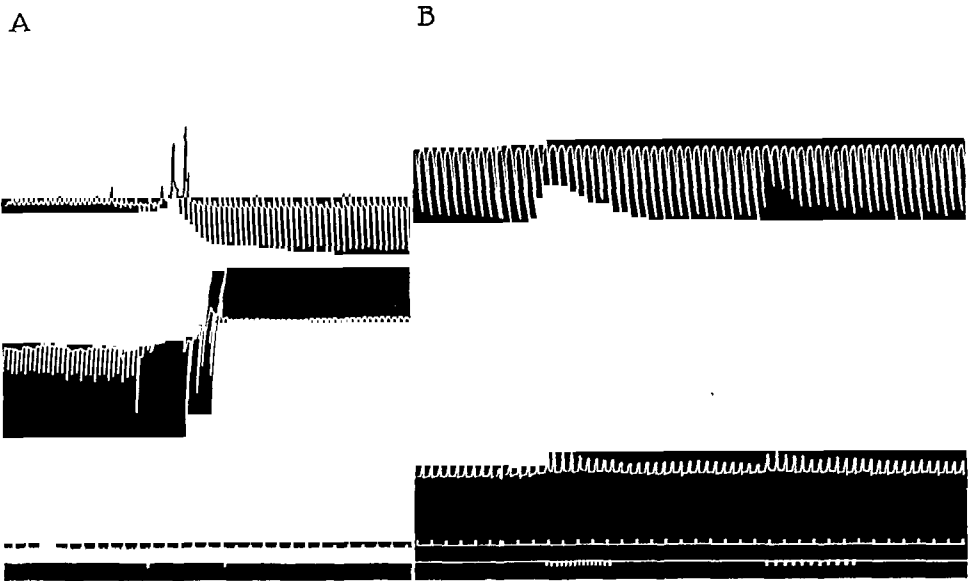


Fig. 4. Cat. Upper record: left, and lower record: right half of the diaphragm.

A. Left spinal semisection at C₂. Between signals, section of the right phrenic.

B. Left spinal semisection at C₂; right phrenic and vagi cut. At signals, short tetani applied to the central end of the cut phrenic; coil distance: 5 cm.

hemidiaphragm; subsequent section of the active phrenic resulted in slight diaphragmatic contractions on the opposite side.

In general, diaphragmatic respiration without costal participation—i.e., in the dogs with complete spinal transection at C₇—was not sufficient for adequate ventilation of the animals. Especially deficient were the dogs in which only the hemidiaphragm on the side of the spinal semisection contracted after severance of the other phrenic; they only survived for short periods.

Afferent stimulation of the cut phrenic resulted in increased and more frequent respirations in 4 out of the 5 animals in which it was tested; in the other dog slight inhibitions were observed. Strong stimuli (coil distance 7 cm. or less) were necessary to elicit these effects.

Asphyxia was produced in 4 animals by occluding the tracheal cannula during 15 to 30 seconds before severing the vagi and the phrenic. It did not result in contractions of the paralyzed hemidiaphragm.

C. *Cats*. Eight animals were studied. In all of them a spinal semisection was performed at C₂ or C₃. This semisection resulted in clear ipsilateral diaphragmatic paralysis in 3 cats only; in the other 5, movements of both hemidiaphragms were recorded, although the autopsy showed a complete semisection in at least 2 of them. The passive movements of a paralyzed hemidiaphragm (e.g., after section of a phrenic) are, however, as a rule very marked in cats. No thorough controls were made to rule out entirely such passive movements or the possibility of a peripheral phrenic crossing (see section A) in these 5 cats. We shall therefore report in detail only the other 3, in which the respiratory hemiplegia was clear.

Section of the vagi and cervical sympathetics did not produce any crossed phrenic effects. Asphyxia was likewise inefficient in this respect. Section of the active phrenic, on the other hand, resulted in crossed diaphragmatic contractions (fig. 4A). The crossing, however, did not occur promptly (cf. section E), but only appeared after a delay of from 10 to 60 seconds. The contractions of the crossed side were quite small, as compared with those of the other side before the section of the phrenic.

The detailed protocol of one of the 3 cats which showed a respiratory hemiplegia is the following. The spinal cord was semisected on the left side between C₂ and C₃, and completely transected between C₅ and C₆. The dorsal roots C₃, C₄ and C₅ were cut on both sides. The cranial nerves IX, X, XI and XII were cut on both sides. The cervical sympathetics were also cut. The right hemidiaphragm was breathing. The right phrenic was then severed, whereupon, after a delay of about 10 seconds the left hemidiaphragm started contracting. The ventilation was not sufficient, however, for the needs of the animal; artificial respiration was given; when after 10 minutes this was stopped the left hemidiaphragm again showed respiratory movements, then gasps, and the cat died.

Afferent stimulation of the cut phrenic nerves (4 animals) inhibited the respiratory excursions of the other side (fig. 4B). This inhibition was only obtained, however, with relatively strong stimuli (coil distance 6 cm. or less), weaker shocks producing no effects.

D. *Guinea pigs*. Three animals were tested. In two of them a right spinal semisection was made at C₂. A typical respiratory hemiplegia ensued. The left phrenic was then cut: no crossed effect was obtained and the diaphragm was fully paralyzed. The left costal respiration persisted but was incapable of maintaining life; the animals died in a few minutes.

In the third guinea pig, besides the right spinal semisection at C₂, a

complete transection at C₇ was made, the dorsal roots C₃, C₄, C₅ and C₆ were cut on both sides and the vagi were severed. The left hemidiaphragm was contracting. Section of the left phrenic resulted in quick death. No contractions of the right hemidiaphragm were observed, although nasal and laryngeal respiratory movements were apparent until the end.

E. Rabbits. Observations were made on 25 animals. In all of them the spinal cord was semisectioned at C₂, producing a respiratory hemiplegia. In 4 a complete transection was also made at C₆ or C₇, paralyzing all costal respiration; the breathing movements of one hemidiaphragm sufficed to keep the animals alive.

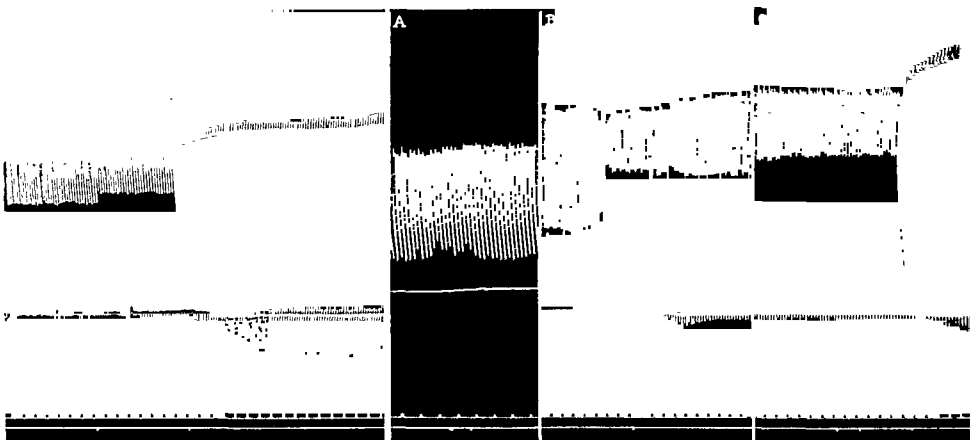


Fig. 5

Fig. 6

Fig. 5. Rabbit. Left spinal semisection at C₂. Upper record: right, and lower record: left half of the diaphragm. Vagi cut. At signals, right phrenic cut, first below C₄, then below C₆.

Fig. 6. Rabbit. Left spinal semisection at C₂. Upper record: right, and lower record: left half of the diaphragm.

- A. Section of the right 6th cervical nerve.
- B. Section of the right 5th cervical nerve.
- C. Section of the right 3rd, then 4th cervical nerves.

Section of the vagi (13 animals), cervical sympathetics and depressors (8 animals) and carotid-sinus nerves (4 animals) failed to produce a crossed diaphragmatic activity.

Section of the active phrenic, whether the vagi were intact (6 animals) or severed (9 animals), but with uncut cervical sympathetics, depressors and carotid-sinus nerves, almost immediately resulted in respiration of the opposite, previously paralyzed hemidiaphragm (figs. 5 and 6). When the entire phrenic was not cut at one stroke, but its several roots were severed successively, the crossing over only occurred when the last root was cut, although paralysis of a considerable portion of the diaphragm resulted from the first sections (cf. fig. 2). Schiff (1894) reported that the

crossing only occurred when the component of the phrenic contributed by C_6 was cut. We failed to confirm this statement, for if the cervical nerves C_3 , C_4 , C_5 and C_6 were cut successively at their emergence from the spinal column, the crossing occurred when C_5 or C_6 was cut if the sections were made in the order stated, but the crossing only occurred on cutting C_4 if the sections were made in the reverse order (fig. 6).

When the whole phrenic nerve was cut at one stroke the crossing appeared immediately. Indeed, the first crossed contraction, though small, occurred usually at the precise time at which the other side would have contracted, had its nerve been intact (fig. 5). A gradual increase of the crossed responses appeared thereafter and in 10 to 20 seconds a steady state was attained.

The method adopted for recording is mainly qualitative. No quanti-

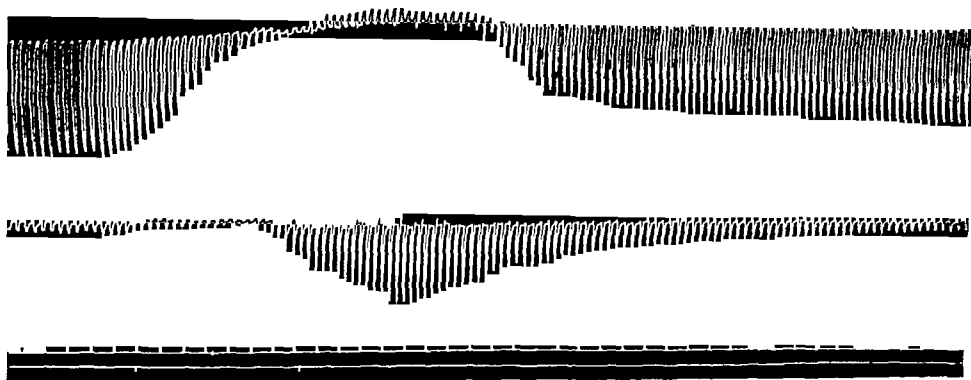


Fig. 7. Rabbit. Left spinal semisection at C_2 . Upper record: right, and lower record: left half of the diaphragm. Vagi cut. Between signals, application of a pledget of cotton soaked with ether to the right phrenic.

tative judgments can be made from the records, therefore, as regards the magnitude of the contractions on the crossed as compared to the opposite hemidiaphragm. It was observed, however, that the costal excursions increased after section of the phrenic, in the animals in which they had not been abolished by a complete spinal transection (cf. fig. 6). Furthermore, no contractions of the crossed slip of the diaphragm inserting at the xiphoid cartilage were detected. It may be concluded, therefore, that the excursions of the crossed hemidiaphragm were not as extensive as those of the originally active side.

Reversible crossed phrenic discharges were readily obtained by applying for short periods pledgets of cotton soaked with ether to the phrenic opposite the spinal semisection, instead of cutting. Figure 7 illustrates a typical example. As many as 7 crossings were obtained in the same

animal by this procedure. The results differed from those elicited by the section of the phrenic as follows. Whether the paralysis of the active hemidiaphragm occurred rapidly (5 to 10 seconds) or very gradually (several minutes), by applying various amounts of ether, the crossed contractions did not appear until practically complete cessation of respiratory activity in the blocked side. Indeed, it was possible by removing the ether opportunely to paralyze almost entirely the diaphragm without the appearance of any crossed contractions.

In contrast with the practical lack of simultaneous contractions of the two sides at the onset of the ether block, during the recovery the direct side usually started contracting before the crossed activity had subsided,

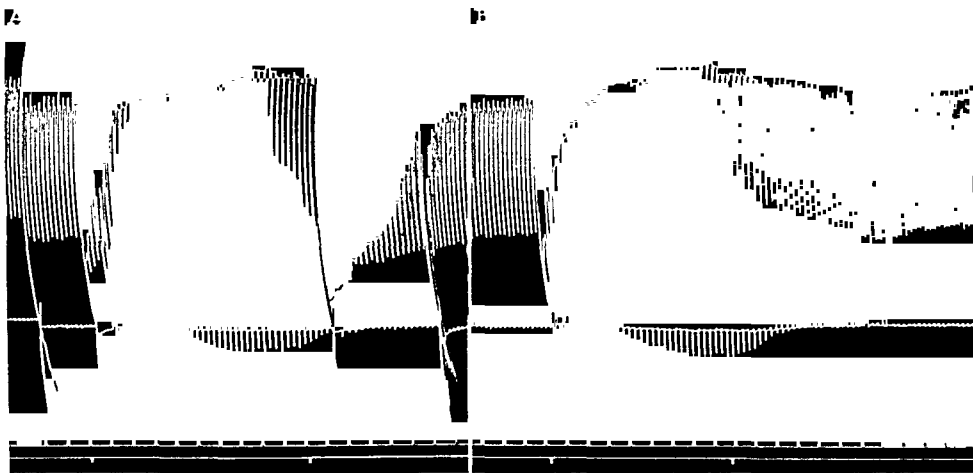


Fig. 8. Rabbit. Left spinal semisection at C_2 . Right C_6 nerve cut at its emergence from the spinal column. Upper record: right, and lower record: left half of the diaphragm. Between the signals, direct current applied to the right phrenic between C_5 and C_6 .

A. Anode cephalad.

B. Cathode cephalad.

so that simultaneous movements were sometimes present for as long as several minutes.

Reversible blocks were also obtained by direct current as follows. The 6th cervical nerve was cut at its emergence from the spinal canal, and a pair of shielded electrodes was placed on the uncut active phrenic between C_5 and C_6 . The direct current was drawn from a battery connected to these electrodes through a potentiometer. The intensity was gradually increased until complete paralysis occurred. The results were in all points identical with those obtained from the ether blocks, previously described. Reversing the current did not modify these results—i.e., it was immaterial for the crossing whether the anode or the cathode was applied cephalad (fig. 8).

In no instance, whether after section of the phrenic or during the ether or direct current blocks, was crossed costal respiration observed.

In 4 rabbits, in which both vagi, cervical sympathetics and depressors were cut before severing the active phrenic or blocking it by ether, the latter procedures did not result in crossed diaphragmatic contractions (fig. 9). In 4 other rabbits, on the other hand, an ether block was applied after severance of the vagi only, with the typical crossed effects; when the cervical sympathetics, depressors and carotid-sinus nerves were subsequently cut, the ether block now was effective in eliciting crossed contractions. These contractions, however, were delayed and smaller after than before severance of the circulatory proprioceptors. An explanation for these apparently inconsistent results will be suggested in the discussion.

Asphyxia was produced by either occlusion of the tracheal cannula

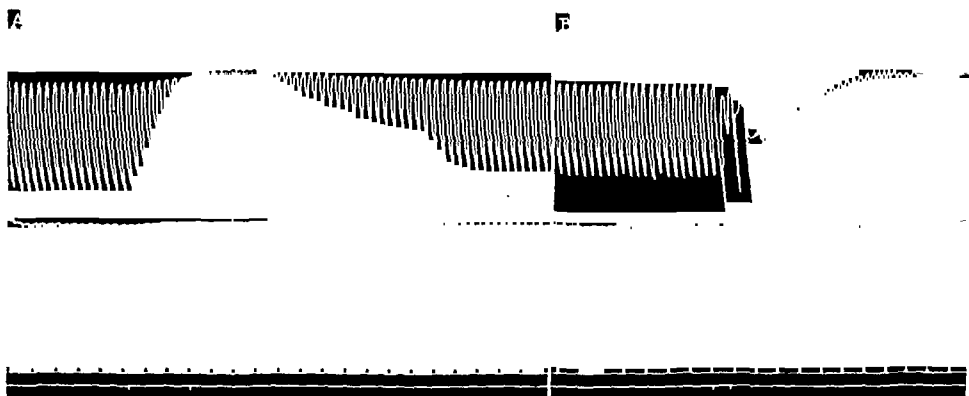


Fig. 9. Rabbit. Left spinal semisection at C_2 . Upper record: right, and lower record: left half of the diaphragm. Vagi, cervical sympathetics, depressors and carotid-sinus nerves cut.

A. Between signals, cotton soaked with ether applied to the right phrenic.

B. Section of the right phrenic.

or connection of it with a rubber balloon containing expired air or nitrogen. Neither procedure elicited crossed diaphragmatic contractions when performed before cutting the active phrenic or blocking it with ether (fig. 10), even if the asphyxia was carried till failure of the respiration. On the contrary, asphyxia did sometimes lead to crossed contractions when applied after an ether block had already elicited responses from the crossed side; these positive results were only obtained when the depressors or carotid-sinus nerves were intact (fig. 11).

Further tests for the rôle of asphyxia in the production of the crossed phenomenon were the following. In 3 rabbits artificial respiration was administered and the thorax was widely opened before section or block of the active phrenic. The ventilation was adjusted to very slightly less than that which would cause apnea, and was kept constant thereafter.

Section of the phrenic again evoked crossed diaphragmatic activity. In 3 other rabbits the phrenic nerves were dissected on both sides between C_5 and C_6 after the spinal semisection at C_2 . The action potentials on the active side were amplified and led to a loud speaker and a cathode-ray oscillograph, so that they could be heard and photographed. Curare was then injected in a dose sufficient to paralyze all respiratory movements. Artificial respiration was administered, and adjusted as before so that nerve impulses were rhythmically discharged through the phrenic on the direct side. The crossed phrenic was still totally inactive in these

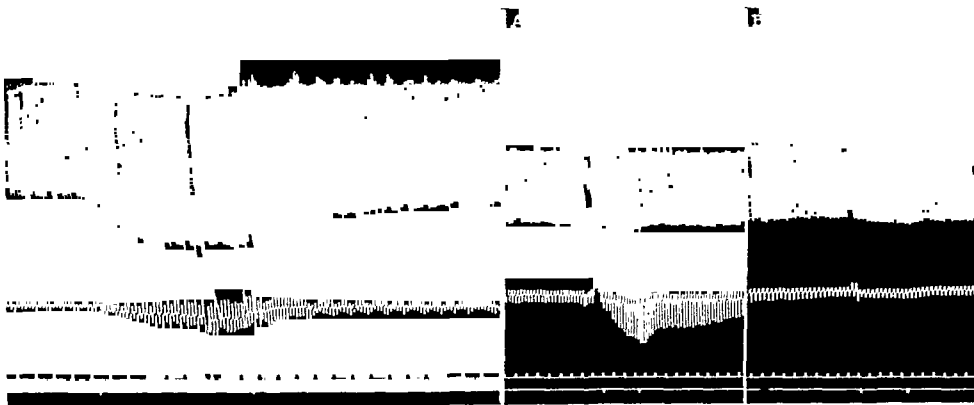


Fig. 10

Fig. 11

Fig. 10. Rabbit. Left spinal semisection at C_2 . Upper record: right, and lower record: left half of the diaphragm. Between signals, rubber balloon containing expired air connected to the tracheal cannula. The movements of the left hemidiaphragm were all unquestionably passive.

Fig. 11. Rabbit. Left spinal semisection at C_2 . Upper record: right, and lower record: left half of the diaphragm. Six reversible crossings were obtained by means of ether blocks in the course of the experiment before these records were made. Vagi, cervical sympathetics and depressors cut.

A. Between signals, rubber balloon with nitrogen connected to the tracheal cannula.

B. Three minutes after A. Carotid-sinus nerves cut in the interval. Between signals, same as A.

conditions. Turning off the artificial respiration for 20 to 30 seconds merely intensified the discharges in the direct side, but did not produce any crossed activity. Severance of the active phrenic below the electrodes, with constant artificial respiration, led to bilateral simultaneous discharges—i.e., the crossing occurred and the nerve impulses on the side originally active were not apparently modified. The discharges on the crossed side were not as intense as on the phrenic directly connected to the medulla.

Intermittent peripheral stimulation of the cut phrenic did not inhibit the crossed respiratory impulses (fig. 12C) unless marked hyperventilation

was produced, and consequent apnea. When this hyperventilation was prevented by letting the animal breathe into a rubber balloon with expired air, no inhibition could be elicited.

Strong afferent stimulation of the cut phrenic between C_5 and C_6 was usually without influence on the respiratory activity of the opposite hemidiaphragm; in only two instances out of seven did a questionable inhibition appear. The possibility of afferent fibers in the phrenic playing a rôle in the appearance of the crossed phrenic phenomenon was further tested by severance of the dorsal roots as follows. In one rabbit a left spinal semisection was made between C_2 and C_3 and the right dorsal

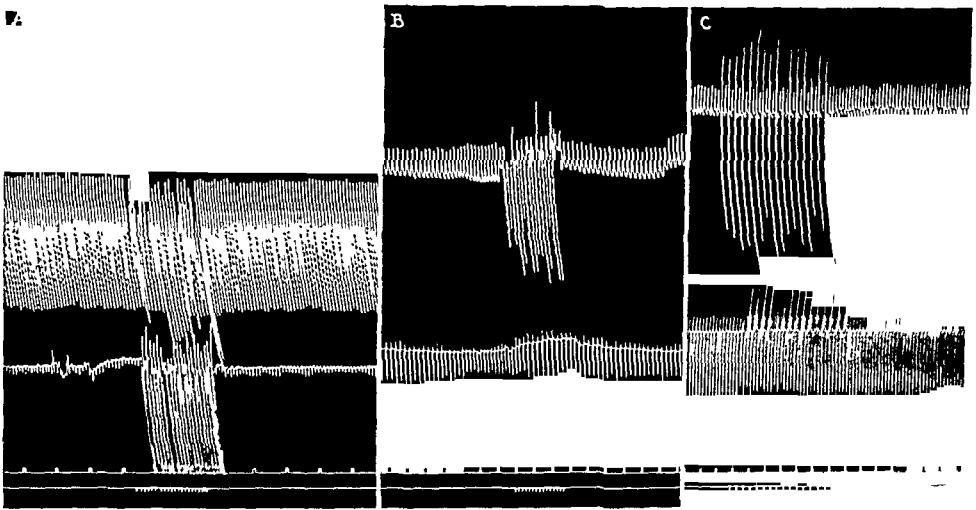


Fig. 12. Rabbit. Left spinal semisection at C_2 . Vagi cut. Upper record: right, and lower record: left half of the diaphragm.

A. At signals short tetani applied directly to the left hemidiaphragm.

B. After cutting the right phrenic. At signals short tetani applied directly to the right hemidiaphragm. The movements of this hemidiaphragm not produced by the stimuli are passive upward excursions produced by the activity of the ipsilateral costal respiration.

C. Short tetani applied to the peripheral end of the cut right phrenic.

roots C_3 , C_4 , C_5 and C_6 were cut. A complete spinal transection between C_6 and C_7 was also performed. Only the right hemidiaphragm was contracting. Section of the right phrenic immediately resulted in paralysis of the right and respiration of the left hemidiaphragm. In another rabbit a similar preparation was made, but in addition the vagi were cut before severing the phrenic. Asphyxia was tested, and failed to produce contractions of the paralyzed hemidiaphragm, while section of the phrenic elicited typical results.

Other tests for afferents in the phrenic nerves were made by stimulating intermittently the paralyzed hemidiaphragms while the other side was

breathing. Such a stimulation did not inhibit either the half opposite the spinal semisection, when the phrenics were intact, or the crossed activity on the side of the semisection after the opposite phrenic had been cut (fig. 12A and B).

F. *Woodchucks*. Although only two woodchucks (*marmota monax*) were successfully studied, the results are reported because they were quite clear and because of their interest in comparing them with the other species.

In one of the animals a spinal semisection was made at C₂, which resulted in an ipsilateral respiratory hemiplegia. Section of the vagi elicited only slight typical effects. Section of the active phrenic was promptly followed by crossed diaphragmatic respiration. Neither central nor peripheral stimulation of the cut phrenic with various frequencies and intensities yielded inhibition of the crossed respiratory movements.

In the other woodchuck, in addition to a spinal semisection between C₂ and C₃, a complete transection was made between C₆ and C₇ and the dorsal roots C₃, C₄, C₅ and C₆ were cut on both sides. Only the hemidiaphragm on the side opposite the spinal semisection was contracting. An ether block was applied to the active phrenic, which resulted in a reversible crossing in all points similar to those obtained in the rabbits. Subsequent section of the phrenic after recovery from the ether block led to a permanent crossing of the impulses to the diaphragm. In this woodchuck also neither peripheral nor central stimulation of the cut phrenic inhibited the crossed diaphragmatic activity.

DISCUSSION. The differences between the several species studied are summarized in table 1. The monkeys and guinea pigs on the one hand, and the cats, rabbits and woodchucks on the other, behaved similarly. The dogs differed from all the other species. Obviously, with such heterogeneous results a phylogenetic analysis would not be fruitful. The attempt to systematize these species differences in terms of the relative importance of diaphragmatic and costal respiration, as judged from the present data, leads likewise to inconsistent conclusions (table 1). Thus the dogs and rabbits, in which crossed phrenic effects are present, have apparently opposite relative importance of the costal and diaphragmatic respirations.

The dogs stand out as the unique species in this series, in which severance of the vago-sympathetic-depressor nerves led to crossed respiratory discharges. Quantitatively the slowing and deepening of respiration consequent upon double vagotomy was also greater in the dogs than in the other species. The larger vagal regulation of the respiratory activity of dogs may perhaps be correlated with other biological features of this animal, such as its special adaptation for running. We must, however, leave the phylogenetic aspect of the problem for further investigation.

Contradictory reports have appeared in the literature concerning the question of the effects of a spinal semisection above C_3 on diaphragmatic respiration. While some observers affirm that such a semisection leads to a hemiparesis, others state that both halves of the diaphragm may continue breathing (see Cordier and Heymans, 1935, for references). Indeed, this persistence of contractions on the semisected side was once looked upon as a strong argument in favor of the existence of spinal respiratory centers.

In some species, e.g., the dogs and cats, the passive movements of a paralyzed hemidiaphragm, produced by the activity of the other hemidiaphragm and of costal respiration, are so marked that it is quite difficult, even by careful direct observation, to distinguish passive from active changes (cf. Schiff, 1894). One of the controls which Langendorff (1887) used to establish this distinction was to cut the phrenic on the side opposite the semisection, obviously a poor control in view of the knowledge acquired since. A sure method, which has not been used, however, would be to record the muscle action-potentials.

Another source of error which has usually not been duly controlled is the possibility of a peripheral crossing, such as that encountered in one monkey in the present observations (p. 498). With the exception of some of the cats (section C), on which the controls were not made to decide whether a peripheral crossing occurs or not, all the other observations made agree in showing that spinal semisection results in respiratory hemiparesis, and that in the monkeys and guinea pigs there probably is no crossed phrenic spinal path. As regards the cats, Henderson and Craigie (1936) have also recently reported respiratory hemiplegias on spinal semisections.

Crossed phrenic activity appeared in the dogs (figs. 2 and 3), the cats (fig. 4), the rabbits (figs. 5, 6, 7 and 8) and the woodchucks (section F). In the dogs, although severance of the vagi usually produced contractions of the paralyzed hemidiaphragm (fig. 3A), the fact that cutting the phrenic increased this activity (fig. 3B), and the instance in which cutting the vagi did not elicit a crossing, while section of the phrenic did (section B), indicate that there probably exist in this species phrenic spinal arrangements similar to those of the cats and rabbits. The masking vagal effects and the irregularity of the results obtained, however, make this animal unsuitable for the analysis of the phenomenon. In the cats, likewise, the results were not consistent (section C) and the effects, when present, were not very striking. For these reasons the majority of the controls were carried out in rabbits, and we shall now concern this discussion mainly with them.

When the active phrenic is cut after the spinal semisection at C_2 several effects ensue. First, there may appear a certain degree of asphyxia, particularly when the costal respiration has been eliminated by the spinal

transection at C₇. Secondly, afferent impulses in the phrenic, arising at the active hemidiaphragm, might be interrupted by the section. Finally, since the paralysis of the corresponding hemidiaphragm entails changes in the expansion of the ipsilateral lung, or a cessation of this expansion when the costal respiration was eliminated, afferent impulses arising in the lung might also be interrupted by the section. These afferent impulses would travel in the nerves distributed to the lungs—the vagus and the sympathetic.

Undoubtedly relative asphyxia after section of the phrenic must have increased the number of discharges from the respiratory center in the medulla, particularly when the depressors and carotid-sinus nerves were intact. But that these increased discharges were not sufficient to elicit the crossed phrenic discharges was shown by the following controls. Asphyxia produced before severing the phrenic failed to evoke contractions of the paralyzed hemidiaphragm (fig. 10). And when the phrenic was cut while constant artificial respiration was applied after opening the thorax or under curare (p. 505), the crossing over occurred, although the discharges of the respiratory center were not modified by the section. We should therefore consider asphyxia as a probable adjuvant factor in the production of the crossed phenomenon, but not as a sufficient cause of it. The cases in which asphyxia did result in crossed contractions, when applied after previous blocks of the phrenic by ether (fig. 11), will be discussed below.

That the crossed effects are not due to the interruption by the section of a stream of inhibitory afferent impulses in the phrenic nerve is shown as follows. The phenomenon was not elicited by section of the corresponding dorsal roots (sections C, E and F), but readily occurred thereafter on cutting the remaining ventral root fibers. The possibility might still remain that these ventral root fibers include afferents, but central stimulation of the cut phrenic failed to stop the respiration of the opposite side (sections B, C, E and F). Similarly, direct stimulation of the paralyzed hemidiaphragm homonymous to the spinal semisection before cutting the phrenic, which should activate the corresponding afferents, failed to inhibit the respiration of the active side (fig. 12B).

Afferent phrenic impulses elicited by passive movements from the paralyzed hemidiaphragm before section of the active phrenic were eliminated as a source of inhibition in the woodchuck in which the corresponding dorsal roots were cut (section F), and by the controls in which the peripheral end of the cut phrenic was stimulated (fig. 12C). Such stimulation did not inhibit the crossed respiration.

Inhibitory afferent impulses in the vagi, arising at the lungs, were eliminated by section of these nerves (sections C and E). Inhibitory impulses reaching the centers by afferents in the sympathetic nerves could not have

been at play when the spinal cord was transected at C₆ or C₇ (sections B, C, E and F). Changes in such afferent impulses were further eliminated when the phenomenon appeared while constant artificial respiration was administered either with the thorax widely opened or after curare (section E; cf. Schiff, 1894; Porter, 1895).

Indeed, the time course of the crossed effects on application of the reversible ether or direct current blocks to the active phrenic (figs. 7 and 8) is such as to exclude *per se* both asphyxia and afferent impulses as causes of the crossing. For if either or both factors were at play the crossing should be gradual, when the paralysis is also gradual, and instead, the crossed side only enters into activity after the other side is almost completely paralyzed, regardless of the time necessary for this paralysis, and if the ether block is not complete no crossing occurs (section E).

We are led, therefore, to conclude that severance or block of the phrenic motor fibers is the necessary and sufficient cause of the crossed phrenic effects under the experimental conditions adopted. It is unlikely that a persistent antidromic bombardment of the phrenic motoneurons should be produced by the section, for the peripheral end did not discharge continuously, since the diaphragm was paralyzed on that side. The positive results of the ether and direct current blocks are further evidence against such a possibility. It appears, therefore, that the phenomenon reveals a property of neurones which differs in quality from that of conducting nerve impulses.

It was stressed in the description of the results that the events during the recovery from the ether or direct current blocks differ as a rule from those at the onset of the block (section E). While at first the crossed side does not start contracting until the originally active half is almost entirely paralyzed, during the recovery from the block the originally active side commences usually to contract long before the crossed responses have subsided. Indeed, this subsidence may take a very long time, for after an ether block had disappeared the records were never quite identical with those obtained before the application of the ether. It may be concluded that these data reveal another property of the nervous system, qualitatively different from conduction of nerve impulses. The opening of the new, hitherto unused path by the block alters the properties of the neurones or synapses involved so that they now tend to remain in use for relatively long periods of time, even though the original conditions have been practically reestablished.

This second property accounts satisfactorily for the two apparent inconsistencies observed. Asphyxia only elicited crossed contractions when a previous ether block had been applied (fig. 11)—i.e., when the crossed path had been opened at least once. Conversely, the ether block only failed to produce crossed contractions when applied for the first time after

severance of the vagi, sympathetics, depressors and (in some animals) carotid-sinus nerves (fig. 9)—i.e., when the path had not been opened before this severance—but was effective even after these nerve sections if the path had been originally opened by an early block.²

Any explanation of the crossed effects which postulates a general property of the central nervous system, without consideration of special paths, such as that of Barcroft, quoted in the introduction, should be rejected, for the phenomenon is not present in all species (table 1). Schiff's (1894) statement, that the section of one phrenic specifically enhances the activity of the opposite phrenic, is really descriptive, not explanatory. Porter's interpretation (see introduction) clearly violates the all-or-none nature of nerve impulses and is not supported by observations such as those of Gasser and Newcomer (1922), Adrian and Bronk (1928), and our own (p. 505), which demonstrate that the cut phrenic does not cease to conduct respiratory impulses.

In attempting to formulate a working hypothesis for the properties of neurone paths which the data reveal, the following considerations are pertinent. An hypothesis involving only unineuronal changes from the section or block will be preferable because simpler than one postulating influences on other neurones than those cut. It does not appear plausible, furthermore, that such changes of other neurones should occur without some causal change in the cut motoneurones. The assumption has been made (Ramón y Cajal, 1909; Eccles and Sherrington, 1931; Lorente de Nó, 1935) that neurones are not polarized as regards conduction of nerve impulses—i.e., that an impulse set up anywhere will travel throughout the cell, including the axon, perikaryon and dendrites. There is no direct evidence, however, for such an assumption. Most of the experiments which demonstrate bidirectional conduction have been made on axons; the properties of typical dendrites are very little known.

To account for the phenomena under discussion in terms of nerve impulses transmitted by the cut motoneurones we must postulate either *a*, that some nerve impulses, discharged continuously from the phrenic motoneurones on the intact or direct side to the crossed phrenic nuclei (e.g., by a branch of the corresponding axons) increase in magnitude after the section of the phrenic (e.g., as a consequence of electrotonus); or we may assume *b*, that some branches, axonic or dendritic, of the motoneurones are not accessible to cellulifugal nerve impulses until the phrenic is cut or otherwise blocked.

The suggestion *a* does not seem probable for the following reasons.

² Experiments performed since this paper was sent to press demonstrate that a crossing may occur on cutting the active phrenic after severance of the vagi, sympathetics and depressors, even if no previous ether block was applied. The reason for the lack of crossing in the four rabbits reported here remains obscure.

Although a section of the motor fibers in the phrenic might change the magnitude of the nerve impulses through an electrotonic effect due to the injury potential, anesthetic blocks influence only negligibly the resting potential of nerve fibers (Bishop, 1932). Furthermore, the fact that reversing the direct current blocks (fig. 8) did not change the results argues against an explanation based on electrotonus. The direct evidence available does not support the suggestion, either, for Adrian and Bronk (1928) did not find any significant change in the action potentials recorded from the phrenic before and after peripheral section.

We might then assume, according to suggestion *b*, that the dendrites of the phrenic motoneurons have normally only unidirectional conduction (Gad, 1884), and that blocking the axon makes them by some unknown means capable of cellulifugal conduction, whereupon they can activate the opposite phrenic nuclei, with which they would be assumed to have synaptic connections. That dendrites may activate other neurons was a view first defended, later rejected by Ramón y Cajal (*loc. cit.*), but the question may still be considered open, for the evidence against such synapses is only negative.

Many other working hypotheses are possible, which would not be based on conduction of nerve impulses by the cut phrenic motoneurons. With the data on hand they would be purely speculative. The one developed is suggested only because it appears to be the simplest. On the basis of such an interpretation the data cast doubt on the assumption that nerve impulses spread normally throughout all parts of the neurons; if they did they should be active before the block or section of the phrenic; furthermore, if they did the antidromic impulses started on central stimulation of the cut nerves should activate the crossed phrenic nucleus, but they do not (sections E and F). It is of course possible, however, that this relatively simple hypothesis will prove inadequate and that changes in other neurons than those cut or blocked do occur—i.e., a change in “potential,” chemical, electrical, or other, in the blocked motoneurons, might permit neighboring dendrites from the opposite side to be activated during the block.

The data obtained under curare (p. 505) lead to the conclusion that for the phenomenon to occur it is necessary to block the axons. A neuromuscular barrier such as that effected by curare is not sufficient to produce the crossing.

The two properties of the nervous system evidenced by the data—the opening of an unused path and the tendency for this path to remain open for relatively long periods—are properties which also appear in other unsolved problems of central nervous functions: the establishment of conditioned reflexes and the persistence of learned patterns. Further analysis will determine whether the similarity is meaningful.

SUMMARY

In animals of several species, under dial anesthesia, spinal semisections at C₂ produced ipsilateral respiratory hemiplegias. Section of the active phrenic led to the prompt appearance of crossed diaphragmatic activity in dogs (section B), cats (section C), rabbits (section E) and woodchucks (section F). No crossed contractions occurred in monkeys (section A) and guinea pigs (section D).

Reversible blocks of the phrenic were obtained by means of ether or direct currents. These gave reversible transient crossed contractions similar to those resulting from the sections (figs. 7 and 8; section F).

The crossed effects in the rabbits, cats and woodchucks are not due to asphyxia (p. 509). They are not due to the interruption of inhibitory afferent impulses in the phrenics or vagi or sympathetics when the directly activated phrenic is cut or blocked (pp. 509, 510).

It is concluded that the crossed phenomenon reveals properties of neurone paths differing in quality from the conduction of nerve impulses: the opening of a new, hitherto unused path, and the tendency for such a path to remain open for relatively long periods of time (p. 510).

It is a pleasure to acknowledge our indebtedness to Dr. Hallowell Davis for suggesting the controls with curare and recording the action potentials of the phrenic nerves. To Dr. Henry G. Schwartz' surgical ability we owe the sections of dorsal roots in the rabbits.

REFERENCES

- ADRIAN, E. D. AND D. W. BRONK. *J. Physiol.* **66**: 81, 1928.
BARCROFT, J. *Features in the architecture of physiological function.* Cambridge, 1934.
BISHOP, G. H. *J. Cell. Comp. Physiol.* **1**: 177, 1932.
CORDIER, D. AND C. HEYMANS. *Le centre respiratoire.* Paris, 1935.
ECCLES, J. C. AND C. S. SHERRINGTON. *Proc. Roy. Soc., B* **107**: 597, 1931.
GAD, J. 1884. Quoted by C. S. Sherrington, *The integrative action of the nervous system.* New Haven, 1906.
GASSER, H. S. AND H. S. NEWCOMER. *This Journal* **57**: 1, 1921.
GIRARD, H. 1890. Quoted by SCHIFF AND PORTER.
HENDERSON, V. E. AND E. H. CRAIGIE. *This Journal* **115**: 520, 1936.
LANGENDORFF, O. *Arch. f. Physiol.* **289**, 1887.
LORENTE DE NÓ, R. *This Journal* **112**: 595, 1935.
PORTER, W. T. *J. Physiol.* **17**: 455, 1895.
RAMÓN Y CAJAL, S. *Histologie du système nerveux.* Paris, 1909.
SCHIFF, M. *Beiträge zur Physiologie.* Lausanne, 1894.

FUNCTIONAL BEHAVIOR OF COELIAC GANGLION CELLS OF THE RABBIT

E. H. INGERSOLL

From the Department of Anatomy, Medical College of Virginia, Richmond

Received for publication July 10, 1936

Of all indicators of nerve cell function the Nissl substance is undoubtedly the most easily observable. A long list of workers from the time of Hodge (1) to the present have reported a definite relationship between the size, arrangement and staining reactions of the Nissl substance and the functional behavior of the nerve cell in the central and autonomic nervous systems (2, 3, 4). Others have demonstrated that this material has a definite identity in the living cell and is segregated in the form of definite groups which constitute the Nissl granules (5, 6). In an effort to obtain further information concerning the behavior of the chromidial substance under normal and experimental conditions the coeliac ganglion cells of a series of pure-bred rabbits were studied. Twenty animals were employed in an examination of normal cells while 15 others were subjected to subcutaneous injections of nicotine. Procedures in the removal of the ganglia, fixing, staining and the method of counting the cells were the same as those used in the study of the albino rat (7).

OBSERVATIONS. *Types of cells.* It is possible, using a classification based upon the distribution and staining reactions of the Nissl substance, to separate the ganglion cells, as in the albino rat (7), into at least nine types. However, in order to facilitate analysis, these types were grouped into three classes which are similar to the three classes described for the albino rat. In the first class of cells the Nissl granules are large, evenly distributed throughout the cytoplasm and take a deep blue stain. The cells of class 2 always have a band of Nissl substance at the periphery of the cytoplasm. Cells with a perinuclear distribution of chromidial material, which were common in the rat in this class, rarely occur in the rabbit. Class 3 cells, like those of class 1, have a diffuse arrangement of the Nissl substance, but the granules are smaller, fewer in number and in consequence the cells stain less deeply. In addition some of the cells exhibit an almost complete lack of chromidial substance which is generally indicative of cellular degeneration or exhaustion.

Eccentric nuclei are a regularly recurring feature in the ganglion cells of the rabbit. In the normal Chinchillas 20 per cent of the cells in class 1

and 3, with 40 per cent in class 2, had peripherally placed nuclei. The other two breeds had even higher percentages of this type of cell.

Multinucleate cells are also common in the autonomic ganglia of the rabbit as in other rodents (8). They comprise from 20 to 30 per cent of all the cells and are found in all 3 classes. Such cells have been reported in man, especially in young persons, by Spiegel and Adolf (9). They state that these cells diminish in number with advancing age and are of the opinion that such cells are able to divide without nuclear changes. They believe that these cells are a part of the reserve system of the sympathetic nervous system. However, in the albino rat only a very few of such cells were seen.

TABLE 1
Cell counts in 3 breeds of normal rabbits

GROUP	BREED	TOTAL NUMBER ANIMALS	PERCENTAGE OF CELLS IN CLASS			TOTAL CELL COUNT
			1	2	3	
1	Havana	7	49.9	32.7	17.2	5428
2	Belted Dutch	5	48.6	36.9	14.3	4003
3	Chinchilla	8	37.7	45.8	16.3	6312

TABLE 2
Cell counts of Chinchilla rabbits injected with nicotine

GROUP	TOTAL NUMBER ANIMALS	DOSAGE IN MGM. PER KGM. BODY WEIGHT	INJECTIONS	PERCENTAGE OF CELLS IN CLASS			TOTAL CELL COUNT
				1	2	3	
4	3	Lethal	1 to 2	40.5	41.3	18.2	3106
5	5	1 mgm. every 30"	5 to 26	37.2	39.5	22.9	4982
6	1	2 mgm. every 30"	5	22.6	50.6	26.8	992
7	1	1 mgm. twice daily	42	35.3	46.6	17.6	1088
8	2	5 mgm. twice daily	30	30.4	50.9	18.7	2038
9	3	1 mgm. twice daily	112	25.1	58.0	16.7	3236

Differential cell counts. A summary of the percentages of cells that occur in each class in the normal rabbit is given in table 1. The number in each class in the Havana rabbit compares favorably with that in the albino rat. In the Chinchilla, and less so in the Belted Dutch, there are fewer class 1 and a greater number of class 2 cells than in the Havana. The percentage of class 3 cells in the three breeds of rabbits and in the albino rat is almost the same.

Langley (10) was one of the first to study the effects of nicotine on the autonomic nervous system but as yet little is known concerning its effect upon the nerve cells. With this fact in mind a group of rabbits were given subcutaneous injections of nicotine (table 2). The distribution of the

classes of cells in those animals receiving nicotine for a few hours or less is, with one exception, similar to that in the normal Chinchilla rabbit. Also the Nissl granules in the ganglion cells of this series were as large and distinct and stained as well as in the normal material. There was but little increase in the number of cells with eccentric nuclei. These observations seem to indicate that nicotine given over a short period of time has little effect on the functional activity of the nerve cell as evidenced by the appearance of the Nissl substance or the position of the nucleus.

Six animals (groups 7, 8 and 9) received injections of nicotine twice daily for two weeks or longer. In these groups there is a gradual decrease in the number of class 1 cells and an increase of class 2 cells in comparison with the normal Chinchilla rabbits. This constitutes a distinct shift from the diffuse, deep staining variety of cells to those with a peripheral band of Nissl substance. The chromidial substance in all three classes of cells often presented a picture of chromatolysis of a moderate or severe degree not unlike that described by Ma (11) for morphinized rats. In addition there is an appreciable increase in the number of cells having eccentric nuclei in all three classes of cells. These facts definitely indicate that nicotine administered for long periods of time produces changes in coeliac ganglia cells, which according to Dolley (2) and others, are evidences of functional activity.

SUMMARY

Based upon the characteristics of the Nissl substance at least three classes of cells occur in the coeliac ganglia of the rabbit. A quantitative analysis was made of these classes in 35 animals, comprising 3 breeds, with a total of over 30,000 cells.

Subcutaneous injections of nicotine given for short periods of time produced, in the Chinchillas, little change from the controls. However, when administered over a period of several weeks there was a shift from cells having an evenly distributed, deep staining Nissl pattern to cells with a peripheral band of Nissl substance. In addition moderate chromatolysis was of frequent occurrence together with an increase in the number of cells having eccentric nuclei. These facts indicate functional activity of a moderate degree.

From 20 to 30 per cent of the cells were multinucleated and a high percentage of cells having eccentrically placed nuclei were encountered in all the types of cells.

REFERENCES

- (1) HODGE, C. F. J. Morph. 7: 95, 1892.
- (2) DOLLEY, D. H. J. Med. Research 25: 285, 1911.
- (3) SAITO, T. Orient. Bull. Neuro-Biol. 1: 1, 1925.

- (4) KUNTZ, A. The autonomic nervous system. 2nd ed. Lea and Febiger, Philadelphia, 1934, p. 49.
- (5) BENSLEY, R. R. AND I. GERSH. Anat. Rec. 57: 369, 1933.
- (6) WEIMANN, W. Ztschr. f. d. ges. Neurol. u. Psychiat. 98: 347, 1925.
- (7) INGERSOLL, E. H. J. Comp. Neurol. 59: 267, 1934.
- (8) CARPENTER, F. W. AND J. L. CONEL. J. Comp. Neurol. 24: 267, 1914.
- (9) SPIEGEL, E. A. AND M. ADOLF. Arb. a. d. neurol. Instit. Wien 23: 67, 1922.
- (10) LANGLEY, J. N. AND W. L. DICKINSON. J. Physiol. 11: 265, 1890.
- (11) MA, W. C. Psychiat. en Neur. Bladen. 38: 374, 1934.

THYROTROPIC EFFECT OF PITUITARIES FROM CRETIN RATS¹

ISOLDE T. ZECKWER

From the Department of Pathology, University of Pennsylvania Medical School

Received for publication July 10, 1936

In a previous paper (8), it was shown that following thyroidectomy in young rats, there occurred conspicuous histological changes in the pituitary. In a few weeks' time there accumulate large amounts of dense hyaline material within basophilic cells. These "thyroidectomy cells" are not "castration cells," because they occur in male cretin rats in which atrophy of gonads does not occur after thyroidectomy, and because when gonadectomy and thyroidectomy are performed at the same time in the same animal both "thyroidectomy cells" and "castration cells" occur in the pituitary and can be distinguished from each other by various morphological differences (10). A large number of rats have been studied since the publication of the above papers, and these cells have been found to occur constantly in all completely thyroidectomized rats and persist throughout the period studied, a year after operation. They also were seen in abundance in the pituitaries of thyroidectomized cats and dogs. Apparently the pituitaries of thyroidectomized rabbits do not show these cells, judging from the descriptions of Bryant (1) and of Marine, Rosen and Spark (6). The rat pituitaries also showed decrease in numbers of acidophiles, sometimes to a striking degree, sometimes only slightly. Factors which determine the fluctuations in the degree of acidophile loss have not yet been discovered.

With such obvious histological changes in the pituitary that are quantitative as well as qualitative, one would anticipate alterations in the production of the various pituitary hormones. A correlation of structural and functional changes might make it possible to ascribe the production of certain hormones to cells of specific type. Changes in various endocrines consequent to thyroidectomy might be due to the altered structure of the pituitary, rather than to any direct effect of the thyroid on other endocrines without the mediation of the pituitary.

It has been shown by a number of investigators that when rats are castrated the pituitaries which contain castration cells secrete into the blood stream an excess of gonadotropic hormone and store within the pituitary

¹ Aided in part by grants from the National Research Council and from the Faculty Research Committee of the University of Pennsylvania.

an excess of this hormone. The hyaline material within the castration cells might be looked upon as gonadotropic hormone which is stored when there is no end organ for it to act upon. Similarly the cells in the pituitary which react to thyroidectomy might be the cells which are producing the thyrotropic hormone, and the hyaline material might represent excess storage of the hormone.

EXPERIMENTAL PROCEDURE. Young immature rats were thyroidectomized, and an equal number of rats of the same sex and age and, in nearly all cases, of the same litter were kept as controls. Adequate thyroidectomy was indicated by 1, retardation of growth; 2, clear-cut changes in ratios of adrenal and gonad weights to kidney weights (9), and 3, increased weight of the pituitary in relation to body weight (8). When the desired time had elapsed after thyroidectomy, varying from 29 to 174 days, each pituitary was removed under aseptic conditions, weighed, suspended in salt solution, and drawn up into a sterile tuberculin syringe through a large bore needle, and injected into the leg muscle of a guinea pig. The guinea pigs were young females from 150 to 200 grams in weight. Injections of pituitaries from thyroidectomized rats were made on 3 or 4 successive days into different legs of the same guinea pig. A second guinea pig received pituitaries from the normal control rats of the same sex as the cretins over the same number of days, and two other guinea pigs were kept under the same environmental conditions in the same cage but received no pituitary injections. All the guinea pigs were killed on the day following the last injection, their thyroids weighed and microscopic sections made. A series of similar experiments was carried out, using in all 16 male cretin rats, 16 male controls, 13 female cretin rats, 13 female controls, 16 injected guinea pigs and 12 control uninjected guinea pigs. One experiment of the series is represented in table 1, which illustrates the method and expresses the comparison of male cretins with litter-mate female cretins, the comparison of cretin with control of the same sex, and also permits comparison of normal males with normal litter-mate females.

Rowland and Parkes (7) have studied carefully the various factors which must be considered in estimating the thyrotropic effect quantitatively. They have recorded data on the response of the guinea-pig thyroid to varying amounts of pituitary substance, the relation of thyroid weight to body weight of the guinea pig and the effect of the time intervals. According to their data the 4 and 5 day period which we chose is a desirable point on the ascending curve, and is long before refractoriness to the injections develops. Any fluctuations of the thyroid due to season, diet and environment was obviated by the fact that in each series of experiments there were two normal uninjected guinea pigs in the same cage, and such controls were taken as the standard of comparison for the injected.

RESULTS. Microscopically an intense hyperplasia was seen resulting from normal pituitary injections. This hyperplasia was manifested by almost complete disappearance of colloid; the cells changed in shape from

TABLE 1

DONOR RATS							RECIPIENT GUINEA PIGS							
Rat number	Sex	Age	Days after thyroidectomy	Body weight	Pituitary weight	Day of injection	Guinea pig number	Sex	Body weight	Thyroid weight	Thyroid/body weight of injected guinea pig = A	Thyroid/body weight of control guinea pig (average)	$\frac{A}{\text{Sum of body weights of rats}} \times 1000 = \text{Index}$	Microscopic hyperplasia
6Q6*	F.	203	165	147	17.9	1st	43	F.	154					
6Z8	F.	205	166	124	12.1	2nd								
7D5	F.	219	168	140	12.0	3rd								
7E8	F.	214	163	139	16.2	4th			149	44.0	1.47	2.7	++	
				550	58.2									
6Q5	F.	203	Intact	172	10.9	1st	44	F.	149					
6Z7	F.	205	Intact	187	10.7	2nd								
7D6	F.	219	Intact	186	9.5	3rd								
7E4	F.	214	Intact	200	9.8	4th			148	34.5	1.21	1.6	++	
				745	40.9									
6Q2	M.	203	165	161	9.4	1st	45	F.	167					
6Z2	M.	205	166	160	10.1	2nd								
7D4	M.	219	147	210	11.2	3rd								
7E1	M.	214	163	158	11.5	4th			164	49.5	1.52	2.2	++	
				689	42.2									
6Q4	M.	203	Intact	245	7.0	1st	46	F.	167					
6Z4	M.	205	Intact	261	7.7	2nd								
7D2	M.	219	Intact	256	6.1	3rd								
7E3	M.	214	Intact	311	9.1	4th			169	53.5	1.68	1.6	++	
				1073	29.9									
							47	F.	141					0
									161	24.1				
							48	F.	192					0
									213	24.9				

* The same alphabetic enumeration of rats indicates litter-mates.

flat to large cuboidal cells; there was an increase in number of cells as indicated by the very cellular appearance, their stratification, and some invagination into the lumen. This was accompanied by intense vas-

cularity seen grossly and microscopically. All of these conditions indicated that the experimental conditions were satisfactory for the demonstration of thyrotropic hormone.

The thyroids of guinea pigs receiving cretin pituitaries showed about the same intense hyperplasia histologically. Sometimes the cretin-injected guinea pigs seemed to show a little more hyperplasia; usually they showed a little less. The uninjected guinea pigs had normal thyroids with abundant dense colloid and flat cells and inconspicuous capillaries. The weights of the thyroids were regarded as a better index of stimulation than the histological appearance. The thyroids of all cretin-injected guinea pigs were increased in weight above those of the uninjected controls. Consequently the cretin pituitary contains abundant hormone. Usually,

TABLE 2

Thyroid/body weight of injected guinea pigs
 Thyroid/body weight of uninjected guinea pigs (average) divided by sum of body
 weights of rats = Index of amount of hormone available per
 gram body weight of rat

GROUP	PITUITARIES INJECTED OF CRETIN RATS	DAYS AFTER THYROIDECTOMY	INDEX	PITUITARIES INJECTED OF NORMAL CONTROL RATS	INDEX
1	1 male, 2 females	118, 46, 29	7.0	1 male, 2 females	5.6
2	3 females	84, 85, 86	6.6	3 females	3.9
3	4 females	165, 166, 168, 163	2.7	4 females	1.6
4	4 females	93, 79, 174, 92	4.0	4 females	1.9
5	3 males	56, 31, 52	5.2	3 males	5.1
6	4 males	91, 44, 99, 108	4.2	4 males	2.8
7	4 males	165, 166, 147, 163	2.2	4 males	1.6
8	4 males	131, 79, 169, 93	3.0	4 males	2.2
	16 rats		(Av.) 4.3	16 rats	(Av.) 3.1

however, the absolute weights of the thyroids of guinea pigs injected with cretin pituitaries were not as great as after normal pituitary injections.

If one considers that the dwarfed cretin rat has available for its small body almost as much hormone as does the larger rat of the same age, then the cretin has available more, not less, hormone for each gram of its body weight. This is expressed mathematically in the next to last column of table 1, where the factor of thyroid increase (A) is divided by the sum of the body weights of the rats whose pituitaries were used, in order to express quantitatively an index of the amount of hormone available for each gram of rat's body weight. It is a matter of judgment whether this calculation is justifiable. If one tentatively does consider the results in this light, then it will be seen in table 2 that this index is greater for the cretins than for the controls in every experiment without exception. The only case (group 5) in which the increase is negligible was an experiment in which the shortest time had elapsed after thyroidectomy. That this is a justifiable

calculation is indicated by the data in table 1 for the normal male and female rats. It will be seen that the normal males totalling 1073 grams caused an absolute weight of thyroid of 53.5 mgm., while the much smaller female rats of the same litters totalling 745 grams, gave a thyroid weight of only 34.5 mgm. Yet presumably with equal basal metabolic rates, there should be equal thyrotropic hormone in the two sexes. If now calculations are made for body weights, it will be seen that the index of both is 1.6, which is reasonable. Since such a calculation is necessary to give reasonable values in normals, it is logical to employ it in comparing dwarfed cretins with controls of normal size.

In table 2 the index is less when 4 pituitaries were injected than when 3 were injected, because the factor of thyroid increase was divided by a larger figure for body weights in the case of 4 rats. The female cretins showed a little more hormone than did the cretin males of the same litter and approximately equal post-operative period (groups 3 and 4 compared with 7 and 8).

DISCUSSION. The cretin pituitaries are increased in size relative to body weight and usually by absolute weight due to the accumulation of hyaline material. Therefore the actual weight of pituitary substance injected was usually greater in the cretins than in the controls. The object of the experiment was to compare the cretin rat with its control irrespective of the weights of the pituitaries. However when calculations were made, each milligram of cretin pituitary contained about as much hormone as each milligram of control pituitary, expressed as index of hormone available per gram body weight of rat. In other words, increased amount of hormone available in the cretin is attained by increase in weight of pituitary.

Since no cell except the basophilic thyroidectomy cell is increased in cretin pituitaries, presumably this is the cell which produces any excess thyrotropic hormone.

The thyroidectomy cells appear histologically like hypersecreting cells, and if it proves correct to use the index discussed, the hypersecretion of thyrotropic hormone by the cretin pituitary is consistent with the histological changes. However, the index of increase does not seem as great as the histologically demonstrable amount of hyaline material would indicate. Perhaps this material is high in protein and contains relatively little hormone for its volume, just as "colloid goiter yields large amount of iodine-deficient thyroglobulin" (3).

It is reasonable to expect hypersecretion of thyrotropic hormone in a hypothyroid animal. When there is a lack of thyroid secretion it is to be expected that there should be a compensatory hyperactivity of the pituitary cells which form thyroid stimulating hormone, which would act upon any remnant of thyroid tissue that was present, this being in accordance with what we see in many compensatory reactions which tend to maintain

equilibrium. The thyroid-pituitary relationship must be a self-limiting process under usual conditions. It has been shown that feeding thyroid extract to rats reduces the thyrotropic effects of their pituitaries to a minimum (4). A reasonable conception would seem to be that when the pituitary stimulates the thyroid to secrete, its thyroxin in turn acts upon the pituitary suppressing its secretion of thyrotropic hormone until a fall in thyroxin results in the pituitary resuming its secretion. In the cretin with no thyroxin there is no inhibition of the pituitary and it continues to secrete unchecked.

Several previous studies of thyrotropic effect of pituitaries from thyroidectomized animals have been made. Hohlweg and Junkmann (4) studied the effect of pituitaries of rats thyroidectomized 5 to 20 days previously. They had 25 to 50 per cent mortality after thyroidectomy. This must have been due to some complicating factor such as parathyroid removal or trauma of recurrent laryngeal nerves; for when we avoided these, no fatalities occurred. In our experience 20 days is too short a time after operation to permit more than the beginning histological changes in the pituitary. They give no weights of injected guinea-pigs' thyroids. Judging from the histological appearance of the thyroid after varying doses of pituitary substance were given, they concluded that there was no increase in thyrotropic effect of thyroidectomized rats' pituitaries.

Houssay, Novelli and Sammartino (5) injected into guinea pigs the pituitaries of adult rats of 200 grams, one month and a half after thyroidectomy. The average weights of the guinea-pig thyroids were 30 mgm. for the uninjected controls, 43 mgm. for those injected with normal rat pituitaries, and 34 mgm. for those injected with pituitaries from thyroidectomized rats, and they concluded that there was no increase in the thyroid stimulating effect after thyroid ablation. They gave two injections of one and a half lobes each and killed the guinea pigs on the 3rd day.

Ch'en and Van Dyke (2) studied rabbits. Since there are so many species differences in the reaction of the pituitary, data on rabbit pituitaries are perhaps irrelevant to the present experiments on rats. They found in 45 ± 5 per cent of paired guinea pigs, the pituitaries of male thyroidectomized rabbits caused greater stimulation of the thyroid than normal pituitaries and in 72 ± 17 per cent of guinea pigs the pituitaries of female thyroidectomized rabbits caused greater stimulation than did normal pituitaries. They felt additional experiments were necessary before conclusions could be definite.

SUMMARY

The thyrotropic hormone content of rats' pituitaries was tested by injecting whole pituitaries on successive days into guinea pigs and noting the effect upon the weight and histological picture of the thyroids.

When thyroidectomy carried out on young rats was adequate (as in-

dicated by dwarfing, increase in weight of the pituitary in relation to body weight, and alterations in ratio of the weights of the adrenals and gonads to the weights of kidneys), the pituitaries contained abundant thyrotropic hormone; but their injection usually caused less increase in absolute weight of the guinea-pig thyroid than did the injection of pituitaries of normal age controls.

If one considers the amount of hormone available to the rat per gram of its body weight, and calculates an index to express this, the index is greater for the cretins than for the controls in every experiment.

REFERENCES

- (1) BRYANT, A. R. *Anat. Record* **47**: 131, 1930.
- (2) CH'EN, G. AND H. R. VAN DYKE. *Proc. Soc. Exper. Biol. and Med.* **32**: 484, 1934.
- (3) HARRINGTON, C. R. *The thyroid gland; its chemistry and physiology.* London, Oxford Press, 1933.
- (4) HOHLWEG, W. AND K. JUNKMANN. *Pflüger's Arch.* **232**: 148, 1933.
- (5) HOUSSAY, B. A., A. NOVELLI AND R. SAMMARTINO. *Compt. rend. Soc. de Biol.* **111**: 830, 1932.
- (6) MARINE, D., S. H. ROSEN AND C. SPARK. *Proc. Soc. Exper. Biol. and Med.* **32**: 803, 1935.
- (7) ROWLAND, I. W. AND S. PARKES. *Biochem. J.* **28**: 1829, 1934.
- (8) ZECKWER, I. T. *Am. J. Med. Sci.* **190**: 145, 1935.
- (9) ZECKWER, I. T. *Proc. Am. Physiol. Soc., This Journal* **116**: 166, 1936.
- (10) ZECKWER, I. T. *Proc. Phila. Physiol. Soc., Am. J. Med. Sci.* **191**: 872, 1936.

THE ORIGIN OF FECAL FAT IN THE ABSENCE OF BILE, STUDIED WITH DEUTERIUM AS AN INDICATOR¹

ARTHUR SHAPIRO, HARRY KOSTER, D. RITTENBERG AND
RUDOLF SCHOENHEIMER

From the Richard Morton Koster Research Laboratory, Crown Heights Hospital, Brooklyn, New York, and the Department of Biological Chemistry, College of Physicians and Surgeons, Columbia University, New York

Received for publication July 11, 1936

When bile is prevented from flowing into the intestinal tract either by a bile duct obstruction or by a bile fistula, the amount of fat in the stools is invariably increased, even though considerable amounts of fat are absorbed (1, 2).

It has also been shown, in starvation experiments on humans, that fat may be secreted into the intestinal tract (3). The important rôle of fat secretion into the intestinal lumen was further experimentally demonstrated on complete bile-fistula dogs kept on a fat-free diet (4). These dogs excreted even more fat than did the control animals, the bile of which ran into the intestinal tract.

This suggests the following question. In humans with complete bile fistula, what fraction of the increased fecal fat represents unabsorbed food fat and how much represents secreted fatty acids?

The new method of labelling physiological compounds such as fatty acids by the introduction of the heavy isotope of hydrogen (deuterium) into the molecule (5), offered the possibility of attacking this problem directly in humans.

Organic molecules in which a part of the hydrogen is replaced by this isotope are, chemically and physiologically, almost identical with their natural analogues. The animal cell is not able to distinguish one from the other. A number of physiological questions have already been solved by the use of such deuterium-containing organic molecules.

If patients with a bile fistula are given a deuterium-containing fat together with the diet, the deuterium content of the fecal fatty acids should indicate how much of this fat has been absorbed, and it can be calculated whether the increase of fecal fatty acids in such patients is derived from the food fatty acids or from secretions.

Two otherwise healthy patients with bile fistulae previously reported

¹ Part of this work was carried out with the support of the Josiah Macy, Jr., Foundation.

upon (6, 7) offered a unique possibility for such experiments. When the bile drainage tube of these patients was opened, no trace of bile flowed into the intestine, as indicated by the complete absence of bile pigments from the stools. On the other hand, when the drainage tube was closed, all bile flowed into the intestine. By alternately opening and closing the drainage tube it was possible to obtain periods with complete absence or presence of bile in the intestine.

The patients were kept on a standard diet, the fat content of which was previously determined. Each experimental period was marked at the beginning and end by giving 0.5 gram of carmine by mouth. Several days were allowed to elapse between periods.

The fat given to the patients contained 4.37 atom per cent deuterium. It was prepared from linseed oil according to Schoenheimer and Ritten-

TABLE 1
Stool fatty acids in bile-fistula humans
(The fat (β 71) contained 4.37 atom per cent deuterium)

PATIENT	PERIOD NUMBER	DURATION OF PERIOD	BILE FLOW	FAT β 71 ADDED PER DAY	TOTAL FATTY ACIDS IN DIET PER DAY	FATTY ACIDS IN STOOLS PER DAY	AMOUNT OF FAT β 71 RECOVERED FROM STOOLS
		days		grams	grams	grams	per cent
D. W.	1	10	Into intestine		8.4	1.23	
D. W.	2	10			8.1	1.99	
D. W.	3	6	Outside	0.75	8.5	8.2	35
D. W.	4	10	Outside		8.1	9.43	
H	5	5	Outside	2.1	6.0	6.22	30
H	6	5	Into intestine		4.0	0.73	

berg (8) and had properties similar to those of olive oil. It was given to the patients in divided doses on bread with their meals on the middle days of the intervals studied.

METHOD OF ANALYSIS. The combined stools for each interval were acidified with glacial acetic acid and dried by powdering with anhydrous sodium sulphate. About 500 gram aliquots were continuously extracted for 24 hours with ether in a modified Clarke extractor. The ether extract was then washed free of acetic acid and sulphate in a separatory funnel with water and dried to constant weight on a steam bath. The dried ether extract was then saponified by refluxing for two hours on a steam bath with 7 per cent potassium hydroxide in 90 per cent methyl alcohol and transferred to a separatory funnel by washing with successive portions of water and ether. More ether and water were added and the unsaponifiable

fraction removed by repeated extraction with ether. The combined ether extract was washed once with water and this water, combined with the original aqueous layer, was strongly acidified with concentrated HCl. The fatty acids were extracted with successive portions of ether, and the combined ether layers were washed with water until neutral to litmus and dried to constant weight on the steam bath.

The deuterium analysis of the fatty acids was done according to Rittenberg and Schoenheimer (9). The results are given in table 1. Melting points and iodine numbers of the fatty acids were determined but, since the results seem to have no bearing on the problem, they have been omitted.

DISCUSSION. Our findings confirm the results of previous workers who showed that when bile is absent from the intestinal tract, the amount of fatty acids in the stools increases. In the periods when bile flowed into the intestinal lumen, the amount of fatty acid in the stools was only a small fraction of that in the diet (15-25 per cent). When the bile ran to the outside, the amount increased to about the same as was given with the diet.

From these findings it might be concluded that no fatty acid was absorbed, and that in these periods the fecal fatty acid represented in its entirety the unabsorbed food fatty acid. That this is not the case is clearly indicated in the experimental periods when the food fatty acid was labelled by the addition of small amounts of deuterium-containing fat (DW3 and H5). While in these periods also the total amounts of fecal fatty acid and food fatty acid were approximately the same, the greater part of the fecal fat must have had another origin. (It contained only 35 and 30 per cent of the deuterium administered with the diet fat.) It can be concluded from these analyses that the remaining 65 to 70 per cent of the diet fatty acid was absorbed and that the greater part of the fecal fat in our patients originated from the fat secreted into the intestinal tract.

SUMMARY

The amount of fecal fatty acid has been compared with the amount of diet fatty acid in two patients with bile fistulae. In the periods when bile did not enter the intestinal tract, the amount of fecal fat was greatly increased. By adding small amounts of deuterium-containing fats to the diet, it was shown that 65 to 70 per cent of the diet fatty acids was absorbed in the absence of bile and that the increase of fecal fat was due to fats secreted into the intestinal lumen.

REFERENCES

- (1) MUNK, J. *Arch. f. path. Anat.* **122**: 302, 1890.
- (2) VON HOESSLIN, H. UND T. KASHIWADO. *Deutsch. Arch. f. klin. Med.* **105**: 576, 1912.
- (3) MULLER, F. *Arch. Path. Anat.* **131**: Suppl. 17, 64, 106, 1893.
- (4) SPERRY, W. M. *J. Biol. Chem.* **71**: 351, 1927.

- (5) SCHOENHEIMER, R. AND D. RITTENBERG. J. Biol. Chem. **111**: 163, 1935.
- (6) KOSTER, H., A. SHAPIRO AND H. LERNER. This Journal **115**: 23, 1936.
- (7) SHAPIRO, A. AND H. KOSTER. This Journal **116**: 317, 1936.
- (8) SCHOENHEIMER, R. AND D. RITTENBERG. J. Biol. Chem. **111**: 175, 1935.
- (9) RITTENBERG, D. AND R. SCHOENHEIMER. J. Biol. Chem. **111**: 169, 1935.

A COMPARISON OF THE CHEMICAL COMPOSITION OF STIMULATED AND RESTING SALIVA OF CARIES-FREE AND CARIES-SUSCEPTIBLE CHILDREN¹

JULIUS WHITE AND RUSSELL W. BUNTING²

From the Department of Biological Chemistry, Medical School and Department of Oral Pathology, Dental School, University of Michigan

Received for publication July 11, 1936

Few studies have been made on the chemical composition of resting saliva. On the other hand, many reports have appeared concerned with the composition of stimulated saliva (paraffin chewing). Little is known as to whether there is any relationship existing between the method of collection of the saliva and the amounts of substances present (1). The variable results of analyses have been discussed by Becks and Wainwright (2), who have emphasized the possibility of explaining these discrepancies by variations in analytical procedure or method of collection of the saliva. Hubbell and Bunting (3) and Hubbell (4), who have reported on the calcium and phosphorus content of stimulated salivas of a large number of children, have been criticized by Becks and Wainwright on the basis that stimulated salivas were used. Wainwright (5) later reported a new technique for the collection of the saliva as well as improved modifications in the determination of calcium. In view of the criticism of Becks and Wainwright and the paucity of comparative data on resting and stimulated saliva, a study was undertaken to obtain such data.

Since we were interested for some time in the possible relationship between the chemical composition of saliva and dental caries, we have studied children with a history of pronounced dental caries and those apparently immune. The subjects used were public school children of known dental history ranging in age from 9 to 16 years. Most of them had been under observation for several years. The children were brought to the laboratory by automobile at 6:30 in the morning. They were previously instructed not to brush their teeth or to partake of foods or liquids. They were allowed to rest 15 minutes more before the actual

¹ A preliminary report of this investigation was presented before the International Association for Dental Research at the 14th Annual Meeting in Louisville, March, 1936.

² Study made as a part of the Dental Caries Research supported by the Rackham Foundation in the School of Dentistry, University of Michigan.

collection of the saliva was started. The resting saliva was collected by having the child tip his head forward with mouth open and allowing the

TABLE 1

The analysis of stimulated and unstimulated saliva of children

Each figure represents an average of the value obtained on analysis of 4 different samples of saliva. The analytical values unless otherwise indicated are expressed as milligrams or cubic centimeters per 100 cc. of saliva. R = resting; S = stimulated.

SUBJECT	AGE	VOLUME		SOLIDS		ASH		CALCIUM		PHOSPHORUS		CARBON-DIOXIDE		pH	
		R	S	R	S	R	S	R	S	R	S	R	S	R	S

Caries-free															
	Yrs.	cc./min.	cc./min.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	cc.	cc.		
1	13	1.05	2.7	243	366	85	133	6.38	5.83	15.3	13.6	12.9	13.6		
2	13	0.32	1.9	415	633	100	130	5.00	4.50	18.1	14.0	17.1	64.0		
3	14	0.59	1.8	324	498	91	115	5.77	4.38	16.5	11.6	24.3	60.9		
4	11	0.39	0.96	312	397	98	158	6.00	5.36	16.5	15.6	32.5	61.1		
5	12	0.49	2.3	377	491	101	115	6.32	5.09	17.5	13.3	11.7	54.2		
6	16	0.28	1.1	520	416	83	96	5.63	3.91	14.2	12.5	7.4	23.2	6.6	7.1
7	9	0.32	0.96	533	560	127	105	6.21	4.63	16.8	12.2	6.4	33.5	6.7	7.5
8	9	0.73	1.3	363	450	101	88	7.30	5.60	15.5	12.4	10.2	29.2	6.7	7.3
9	13	0.63	0.66	427	645	100	120	6.80	5.91	15.1	13.7	5.9	41.0	6.6	7.0
10	15	0.45	2.4	408	571	157	122	7.12	5.43	16.5	14.6	4.4	29.0	6.6	7.1
11	12	0.31	0.59	401	342	113	83	5.40	3.93	15.4	12.8	9.5	23.0	6.5	7.3
12	13	0.19	1.8	560	360	137	92	6.71	5.14	14.1	12.3	20.0	29.8	6.6	6.9
Av.	12.4	0.46	1.52	407	472	108	113	5.79	4.99	16.0	13.3	10.3	40.6	6.6	7.17

Caries-susceptible															
13	11	0.40	1.2	357	451	108	132	4.94	4.57	14.2	10.9	9.9	40.2	6.7	7.3
14	14	0.23	0.63	430	357	91	68	5.30	3.91	16.1	12.9	6.3	24.3	6.8	7.1
15	14	0.35	1.5	335	445	125	135	5.35	5.00	17.1	13.8	5.9	31.8	6.6	7.1
16	13	0.47	1.4	278	293	107	107	5.01	4.68	16.6	13.3	12.0	27.2	6.8	7.2
17	13	0.51	0.96	278	293	92	83	6.02	4.56	14.6	12.8	13.3	31.0	6.6	7.1
18	14	0.41	1.7	333	440	95	110	6.10	5.23	17.2	13.4	9.5	43.3	6.7	7.3
19	12	0.40	1.5	352	542	90	100	6.32	4.99	14.2	11.3	13.5	42.0	6.7	7.4
20	13	0.38	1.5	348	482	53	83	5.17	4.37	17.7	12.9	10.3	43.1	6.5	7.3
21	10	0.35	2.5	425	455	87	90	5.98	4.33	18.4	14.5	5.6	25.2	6.8	7.3
22	13	0.67	1.6	580	522	97	105	6.84	5.70	14.6	10.8	5.6	35.0	6.8	7.4
23	13	0.27	1.5	460	435	117	105	6.49	5.12	16.0	12.3	6.6	40.9	6.7	7.4
24	10	0.45	1.3	298	350	90	86	6.84	5.70	15.6	12.9	21.1	49.2	6.8	7.2
25	9	0.40	1.5	360	370	105	90	6.24	5.01	14.7	13.4	7.7	29.3	6.4	6.8
Av.	11.5	0.40	1.45	373	419	96	99	5.89	4.87	15.9	12.9	9.8	35.6	6.6	7.26

saliva to flow from his mouth without the movement of tongue or jaws. After some practice the subject became adjusted to this position and very

little trouble was encountered in collecting the sample. The saliva was allowed to drip into a small glass funnel resting in a graduated cylinder which contained a layer of paraffin oil. True resting saliva was always water clear and almost totally devoid of cell débris. The movement of the tongue or jaws or yawning caused the saliva to become cloudy. When such was the case, the sample was discarded. The time elapsed and the amount of saliva collected were carefully noted. After 15 to 18 cc. were collected, the subject was allowed to rest 15 minutes, and then a second 15 to 18 cc. sample was collected by paraffin stimulation. On several days the process was reversed, i.e., the stimulated sample was collected first. This was done in order to determine whether or not there was a fatigue factor. Collection of the resting and stimulated salivas on two consecutive days was also tried. Since no significant differences related to the order of collection were found, the procedure finally adopted was the collection of both samples the same morning with a 15 minute rest interval between each collection.

Four 1 cc. samples were removed immediately after collection and the pH (colorimetric) and the carbon dioxide capacity (Van Slyke and Cullen (6)) were determined. The remainder of the saliva collected was centrifuged under oil at a high speed for 30 minutes. Two 2 cc. samples were removed and transferred to a platinum dish and dried at 110° overnight in an electric furnace to determine total solids. The dried material was then ashed in an electric muffle at a temperature between 450 and 500°. After weighing, the ash was dissolved in 1 cc. of HCl (1:3) and the solution was transferred to a 25 cc. volumetric flask and made up to volume. Aliquots of this were used for phosphorus determinations by the method of Fiske and Subbarow (7). Two 6 cc. samples of the centrifuged saliva were transferred to a test tube, treated with 6 cc. of 20 per cent trichloroacetic acid, shaken and allowed to stand 10 minutes. The precipitate formed was removed by filtration and the filtrate analyzed for calcium by the procedure outlined by Wainwright (5) using the Halverson-Bergeim (8) procedure.

Four separate samples were obtained from each individual. There was very little variation between individual samples so that the data in the table represent an average of 4 determinations. Since 25 subjects were used the analyses include a total of 100 samples. It is true that there is a higher concentration of calcium and phosphorus in the resting saliva than in the stimulated but examination of the total solids reveals that with few exceptions (3 in each group) the solids are lower in the resting salivas. The carbon dioxide capacity was lower in the resting than in the stimulated salivas. The values obtained for the stimulated salivas are in accord with those reported by Hubbell and are in the same range as those of Krasnow (9).

Our results do not indicate that the composition of either resting or stimulated saliva is altered by the factor of caries susceptibility.

The cases for this study were selected with the help of Dr. Philip Jay.

SUMMARY

Analyses have been made on the resting and stimulated salivas of 25 children, of whom 12 were caries-free and 13 caries-susceptible.

The earlier work of Hubbell and Bunting on stimulated saliva has been verified.

Resting salivas contained a higher calcium and phosphorus content and a lower carbon dioxide capacity than activated salivas.

There is no apparent relationship between chemical constituents of the salivas studied and dental caries.

REFERENCES

- (1) Cf. Compare KRASNOW, F. *Dental Cosmos* 78: 301, 1936 for a review on the chemical composition of saliva.
- (2) BECKS, H. AND W. W. WAINWRIGHT. *J. Dental Research* 14: 387, 1934.
- (3) HUBBELL, R. B. AND R. W. BUNTING. *J. Nutrition* 5: 599, 1932.
- (4) HUBBELL, R. B. *This Journal* 105: 436, 1933.
- (5) WAINWRIGHT, W. W. *J. Dental Research* 14: 425, 1934.
- (6) VAN SLYKE, D. D. AND G. E. CULLEN. *J. Biol. Chem.* 30: 289, 1917.
- (7) FISKE, C. W. AND Y. SUBBAROW. *J. Biol. Chem.* 66: 375, 1925.
- (8) HALVERSON, J. O. AND O. BERGEIM. *J. Biol. Chem.* 32: 159, 1917.
- (9) KRASNOW, F. *J. Dental Research* 12: 530, 1932.

GASTRIC ACIDITY FOLLOWING PARTIAL GASTRECTOMY AND VAGOTOMY

CHARLES M. WILHELMJ, H. H. MCCARTHY AND FREDERICK C. HILL

*From the Departments of Physiology and Experimental Surgery, Creighton University
School of Medicine, Omaha, Nebraska*

Received for publication July 13, 1936

In a previous publication (1) it was shown that whole stomach pouches from which the pylorus had been removed secreted, on an average, only about $\frac{1}{5}$ to $\frac{1}{6}$ as much acid as whole stomach pouches in which the pylorus was intact. It was also shown that removal of the pylorus and anastomosis of the fundus to the duodenum (Polya type) caused a lowering of acidity which was definitely greater than could be accounted for by the diluting and neutralizing effects of the duodenal secretions entering the stomach. When atropine (0.05 mgm. per kilo) was given intramuscularly to these animals total anacidity resulted in each of six experiments. As a result of these studies it was concluded that the pyloric portion of the stomach is a factor of paramount importance in the intragastric chemical phase of acid secretion and that a large part of the acid secretion which may persist after pyloric removal is cephalic (not psychic) in origin. In the present article we wish to present studies on dogs before and after partial gastrectomy and double vagotomy.

METHODS. A two per cent Liebig's extract test meal containing 15 mgm. of phenol red per liter was used in all experiments (2). The manner of performing the experiments and the management of the animals was the same as that previously described. Four dogs were studied. From 6 to 8 satisfactory fractional gastric analyses were first done on each animal. Both vagus nerves were then cut in the thorax. After recovery the animals were tested to demonstrate the absence of the psychic phase of acid secretion. The pyloric portion of the stomach was then removed and the fundus anastomosed to the duodenum according to the Polya method. A second series of fractional analyses was then performed. The present experiments cover a period up to 3 months after the second operation.

RESULTS. I. *The emptying time of the stomach.* In previous studies following partial gastrectomy without vagotomy (1), it was found, in agreement with the work of others, that the emptying time of the stomach was shortened, usually by about one-half hour. In the present studies

more rapid emptying did not occur, and on all four animals the duration of most of the experiments was the same as before operation (2 hrs.). This finding definitely eliminates a more rapid emptying time as a factor in the results.

II. *Gastric acidity.* In figure 1 the average acidity of the total fluid secretions entering the stomach (lower half) and the average acidity of the gastric contents (upper half) before and after operation are shown on

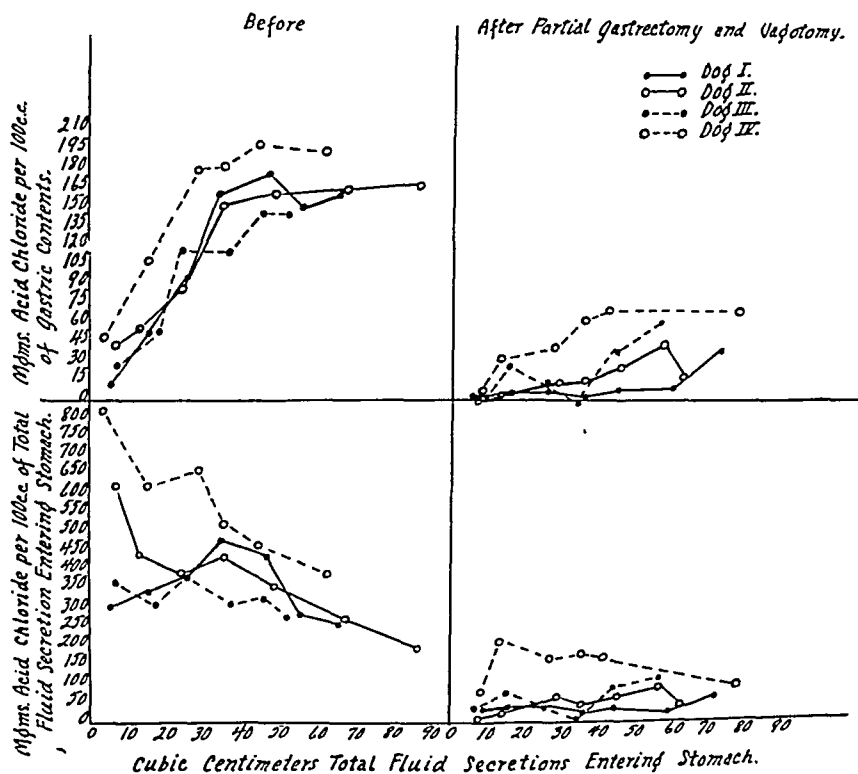


Fig. 1. Shows the average acidity of the gastric contents and of the total fluid secretions entering the stomach on four dogs before and after partial gastrectomy and vagotomy. The samples were grouped according to increasing amounts of total fluid secretions entering the stomach and the average acidity for each group plotted. The analysis is based on 103 half-hour gastric samples obtained before operation and 145 half-hour samples obtained after operation.

each of the four dogs. The samples before and after operation were grouped according to increasing amounts of total fluid secretions entering the stomach, identical groupings being used for the experiments before and after operation, and the average acidity for each group plotted. The acidity of the gastric contents as well as the acidity of the total fluid secretions entering the stomach were profoundly lowered after operation.

In table 1 a few typical experiments are shown on dog II. In the first

four experiments tabulated after operation almost complete anacidity occurred. The acid deficits are due to the fact that since no acid was being secreted, the acidity of the test meal was lowered by neutralization by the non-acid secretions entering the stomach. Experiments in which anacidity occurred were common on all four dogs. The frequency was as follows:

Dog I,	8 out of 16 experiments or 50 per cent
Dog II,	4 out of 14 experiments or 29 per cent
Dog III,	5 out of 14 experiments or 36 per cent
Dog IV,	0 out of 7 experiments or 0 per cent

Hence in 51 experiments after operation 17 or 33 per cent were characterized by anacidity. Dog IV which had the highest preoperative acidity curve which we have ever encountered in a normal dog (due apparently to water absorption (2)) never showed complete anacidity. In the other three dogs anacidity was often encountered even when a second experiment was started immediately after terminating the first experiment (expts. 2 and 3, table 1). In the remaining experiments on all four dogs a low secretion of acid occurred as illustrated in experiments 5, 6, 7, 8 and 9 in table 1.

III. *The response to histamine and to the intestinal phase.* As shown in table 1 a marked secretion of acid occurred in response to histamine administered intramuscularly in dog II. Dog I gave a less marked response, while dog III gave no response. Dog IV was not studied.

During the course of the experiments the animals were purposely placed on a regimen of forced feeding (4 feedings daily) with as much raw ground liver as they would eat, the last feeding being given 24 hours before performing an experiment. After approximately 10 days on this regimen it was observed that the amount of acid secreted was definitely elevated above the previous low value in all four dogs. This effect was sometimes found to be present for as long as 48 hours after the last meat feeding. After discontinuing the forced feeding regimen and placing the animals on the usual single daily feeding of milk and dog biscuits, it required approximately 5 days for the acid secretion to return to its previous low level. This effect is due to either (or both) a general cellular stimulation resulting from the luxus consumption of protein or a greatly prolonged intestinal phase. The latter factor was definitely proven since in all dogs food remnants were found in the duodenal secretions which regurgitated into the stomach even as long as 48 hours after the last meat feeding. The more sluggish intestinal movements probably resulted in food remaining in the upper intestine longer than normal and this in conjunction with the low acidity of the gastric contents entering the intestine, resulted in an unusually prolonged intestinal phase.

TABLE 1

A series of experiments on dog II after partial gastrectomy and vagotomy compared with an average of 7 experiments before operation

P.S.P. PER CENT	MGM. EXTRA ACID CHLORIDE PER 100 CC. GASTRIC CONTENTS	MGM. EXTRA ACID CHLORIDE PER 100 CC. OF SECRETION	TOTAL FLUID	ACID FLUID	NON-ACID FLUID	PER CENT ACID FLUID	TIME	BILE	REMARKS	
89	36	327	11	6	5	55	$\frac{1}{2}$	0	Average of 7 fractional analyses	Before operation
80	82	410	20	14	6	70	1	+		
59	125	305	41	21	20	51	$1\frac{1}{2}$	++		
43	188	330	57	31	26	54	2	++		
93	-4	-57	7	0	7	0	$\frac{1}{2}$	+		
88	-11	-92	12	0	12	0	1	++++		
64	+4	+11	36	0.7	35.3	3	$1\frac{1}{2}$	++++		
64	-8	-22	36	0	36	0	2	++++		
88	-12	-100	12	0	12	0	$\frac{1}{2}$	+++	800. cc. test meal followed by 800 cc. test meal	
62	+10	+26	38	4	34	11	1	+++		
70	-16	-53	30	0	30	0	$1\frac{1}{2}$	++++		
...		
89	-8	-73	11	0	11	0	$\frac{1}{2}$	++++		
88	-14	-117	12	0	12	0	1	++++		
92	-34	-425	8	0	8	0	$1\frac{1}{2}$	+++		
94	-3	-50	6	0	6	0	$\frac{1}{2}$	++		
86	+5	+36	14	1	13	7	1	+++		
72	-4	-14	28	0	28	0	$1\frac{1}{2}$	++++		
64	-6	-17	36	0	36	0	2	++++		
79	+7	+33	21	1	20	5	$\frac{1}{2}$	+++		
66	+20	+59	34	3	31	9	1	+++		
56	+12	+27	44	2	42	5	$1\frac{1}{2}$	++++		
52	-2	-4	48	0	48	0	2	++++		
72	+17	+61	28	3	25	11	$\frac{1}{2}$	++		After partial gastrectomy and vagotomy
62	+31	+82	38	5	33	13	1	++		
62	+26	+68	38	4	34	11	$1\frac{1}{2}$	++		
48	+46	+89	52	8	44	15	2	++		
71	+10	+35	29	2	27	7	$\frac{1}{2}$	+++		
53	+30	+64	47	5	42	11	1	+++		
40	+40	+67	60	7	53	12	$1\frac{1}{2}$	++		

TABLE 1—*Concluded*

	P.S.P. PER CENT	MGM. EXTRA ACID CHLORIDE PER 100 CC. GASTRIC CONTENTS	MGM. EXTRA ACID CHLORIDE PER 100 CC. OF SECRETION	TOTAL FLUID	ACID FLUID	NON-ACID FLUID	PER CENT ACID FLUID	TIME	BILE	REMARKS
82	+2	+11	18	0.3	17.7	2	$\frac{1}{2}$	$\frac{1}{2}$	+++	900 cc. test meal followed by 700 cc. test meal
59	+23	+56	41	4	37	10	1	1	+++	
38	+18	+29	62	3	59	5	$1\frac{1}{2}$	$1\frac{1}{2}$	+++	
...	
83	-2	-12	17	0	17	0	$\frac{1}{2}$	$\frac{1}{2}$	++++	
56	+46	+105	44	8	36	18	1	1	++++	Intestinal phase
85	+14	+93	15	2	13	13	$\frac{1}{2}$	$\frac{1}{2}$	++	
78	+72	+328	22	12	10	55	1	1	+++	
62	+64	+169	38	11	27	29	$1\frac{1}{2}$	$1\frac{1}{2}$	+++	
54	+88	+191	46	15	31	33	2	2	++	
76	+19	+79	24	3	21	13	$\frac{1}{2}$	$\frac{1}{2}$	+++	1 mgm. histamine
56	+96	+218	44	16	28	36	1	1	+++	
38	+142	+229	62	24	38	39	$1\frac{1}{2}$	$1\frac{1}{2}$	+++	1 mgm. histamine
20	+238	+298	80	40	40	50	2	2	++++	

The responses to histamine and to the intestinal phase show that the acid secreting glands were capable of responding to an adequate stimulus in a normal manner.

IV. *The rôle of the duodenal secretions in producing the lowered acidity.* The method of evaluating the part played by the duodenal secretions in lowering the acidity of the total secretions entering the stomach has been described in detail in a previous article (1) and will only be briefly outlined here. Average curves for the *total fluid*, *non-acid fluid* and *acid fluid* entering the stomach before and after operation were plotted on coördinate paper and the areas under the respective curves determined by means of a standardized planimeter and converted into terms of cubic centimeters of the various fluids. These values for the four dogs before and after operation are shown in table 2. It is seen that in dogs I and IV there was a slight increase in the amount of the total fluid secretions entering the stomach after operation which is considerably less than was previously found after partial gastrectomy without vagotomy (1). In dogs II and III the amount of total fluid entering the stomach after operation was slightly less than before operation but in spite of this the acid fluid was 44 per cent lower than before operation. This finding definitely eliminates an increase in the duodenal secretions entering the stomach as the

sole cause of the lowered acidity after operation in these two dogs and shows that considerably less acid fluid must have been secreted.

Three calculations were previously described (1) which were used to determine the exact rôle of the duodenal secretions in producing the lowered acidity. These calculations and their application to the present experiments are as follows:

Calculation I. In this the increase in total fluid entering the stomach after operation is assumed to be due to duodenal secretions. The neutralizing effect of the increased duodenal secretions is ignored but a calcu-

TABLE 2

Shows the average composition of the total fluid secretions entering the stomach in four dogs before and after partial gastrectomy and vagotomy

The values were determined as explained in the text

DOG	BEFORE	AFTER
I	Total fluid = 105 cc. Non-acid fluid = 46 cc. Acid fluid = 59 cc. Per cent acid fluid = 56	Total fluid = 113 cc. Non-acid fluid = 108 cc. Acid fluid = 5 cc. Per cent acid fluid = 4
II	Total fluid = 99 cc. Non-acid fluid = 44 cc. Acid fluid = 55 cc. Per cent acid fluid = 56	Total fluid = 97 cc. Non-acid fluid = 85 cc. Acid fluid = 12 cc. Per cent acid fluid = 12
III	Total fluid = 74 cc. Non-acid fluid = 34 cc. Acid fluid = 40 cc. Per cent acid fluid = 54	Total fluid = 73 cc. Non-acid fluid = 66 cc. Acid fluid = 7 cc. Per cent acid fluid = 10
IV	Total fluid = 103 cc. Non-acid fluid = 21 cc. Acid fluid = 82 cc. Per cent acid fluid = 80	Total fluid = 108 cc. Non-acid fluid = 79 cc. Acid fluid = 29 cc. Per cent acid fluid = 27

lation is made to determine how much lowering of acidity could be brought about by dilution alone. In dogs II and III this calculation is unnecessary, since the per cent of acid fluid is 44 per cent lower after operation with no increase in total fluid. In dogs I and IV the per cent of acid fluid after operation is 48 and 49 per cent lower than the diluting effect of the increased total fluid could account for. In previously reported studies after partial gastrectomy without vagotomy (1) the acidity was from 32 to 40 per cent lower than calculated.

Calculation II. In this the increase in total fluid entering the stomach after operation is considered to be due to duodenal secretions. In addition

to determining the diluting effect, as in calculation I, the neutralizing effect is also evaluated by assuming that the duodenal secretions have an average alkalinity of 0.04 normal and adding the amount of acid fluid that could have been neutralized to the amount observed. In dogs II and III the calculation is unnecessary. In dogs I and IV the per cent of acid fluid after operation is 46 and 48 per cent lower than could be accounted for by both the diluting and neutralizing effects of the increased duodenal secretions entering the stomach. After partial gastrectomy without vagotomy (1) the values ranged from -25 to -38 per cent.

Calculation III. In this allowance is made for the neutralizing effect of the *non-acid fluid* in the gastric samples both *before* and *after* operation by considering it to have an alkalinity of 0.04 normal and adding the acid that could have been neutralized to that observed. If the total fluid entering the stomach after operation is increased the diluting effect is also evaluated as in calculation I. When this calculation was applied to the present series of experiments on four dogs the acid fluid was found to be 36, 35, 37 and 38 per cent lower than could be accounted for. In previous experiments after partial gastrectomy without vagotomy the values ranged from -20 to -32 per cent.

IV. *The consistency of the gastric contents.* After operation the consistency of the gastric contents in dogs I, II and III underwent a profound change due to the presence of large amounts of mucus of about the consistency of raw egg albumin. This was more pronounced in the present series than in partial gastrectomy without vagotomy. The viscosity was often so great that considerably difficulty was experienced in pipetting the samples. There was a very evident correlation between the amount of mucus and the amount of acid secreted. In experiments with anacidity the amount of mucus was very great but when the acid secretion was high (in response to histamine or the intestinal phase) the mucus was often not detectable by gross examination or was greatly reduced. In vitro experiments showed that this mucus was soluble in N/10 hydrochloric acid. This change in the consistency of the gastric contents was not previously observed in whole stomach pouches from which the pylorus has been removed even when the acid secretion was very low (1).

DISCUSSION. The acid secretion following partial gastrectomy and vagotomy is definitely less than was previously found after partial gastrectomy alone (1) thus confirming the statement that part of the acid secreted after partial gastrectomy may be due to the cephalic phase. Studies were not made after vagotomy alone but the work of other investigators has shown that the decrease while definite is not marked, hence most of the lowering observed in the present experiments was due to pyloric removal and consequent interference with the intragastric chemical phase of acid secretion. The cause of the slight secretion which often

occurred is not clear. Since the acid secreting glands were fully capable of responding to adequate stimuli it is quite possible that a summation of several minimal stimuli such as mechanical distention, presence of duodenal secretion in the stomach, etc., were capable of exciting a low rate of secretion. The intestinal phase may also have been active even when the usual single daily feeding of milk and dog biscuits was given.

The increased sensitivity of these animals to the intestinal phase is important and suggests that some of the confusion in the literature on partial gastrectomy may be due to the failure to eliminate the intestinal phase. Unpublished experiments (3) have shown that the presence of acid gastric contents in the intestine definitely inhibits the intestinal phase of acid secretion hence it is quite likely that the low acidity of the gastric contents after partial gastrectomy renders the intestinal phase more pronounced than in normal animals.

Priestly and Mann (4) have suggested that the more rapid emptying time of the stomach which usually occurs after partial gastrectomy is a factor in producing the lowered acidity. Since a more rapid emptying time did not occur in the present experiments, it can be dismissed as a factor of importance.

The average increase in the total fluid secretions entering the stomach after operation was very small in two animals (5 and 8 per cent) while no increase occurred in two others, yet in all four animals the average acidity of the gastric secretions and gastric contents was profoundly lowered. This finding in itself definitely eliminates an increase in duodenal secretions entering the stomach as the sole cause of the lowered acidity after partial gastrectomy. A more detailed analysis shows that the average acidity of the gastric secretions was from 35 to 49 per cent lower than could be accounted for by the diluting and neutralizing effects of the duodenal secretions.

The presence of the very viscid mucus noted in these and previous experiments has to our knowledge not been described before, possibly because of the frequent use of a meat test meal which would obscure it. When large amounts of acid were secreted in response to histamine or the intestinal phase the mucus was not observed possibly because it is soluble in acid. This fact suggests that the mucus is a *result* and not the *cause* of the low acidity. If this viscid mucus occurs in the human stomach after partial gastrectomy, it may be an important factor in hastening the healing and in preventing the recurrence of peptic ulcer.

The acid values given in the present paper are for *total acid*. The gastric samples were, on several occasions, tested for so called "free acid" with Topfer's reagent but were always negative. It is important to note that in the four animals studied the average *total acidity* of the gastric contents after operation was always definitely below the threshold

value of *free acid* necessary for the digestion of living tissues (97 to 146 mgm. of acid chloride per 100 cc.) as determined by Dragstedt (5). It is thus quite likely that experimental jejunal ulcer would not have occurred in these animals following the Mann-Williamson operation.

The gastric acidity curve in response to a Liebig's extract test meal in partially gastrectomized animals before and after double intrathoracic vagotomy, constitutes a new preparation which may prove useful in studying the magnitude of the cephalic (not psychic) influence on gastric acidity. The evidence indicates that it may vary considerably in different animals.

The duration of the lowered acidity was not specifically studied but it was shown to persist for at least three months with no evidence of a return to normal. This result is not in agreement with the findings of Shapiro and Berg (6) who noted a beginning return to normal in from 12 to 33 days and complete return in from 3 to 8 weeks. It should be noted that these investigators attempted abdominal vagotomy, which in the dog can almost never be complete. When even a few fibers are left intact the effect of vagotomy may not be evident (7).

SUMMARY

1. Following partial gastrectomy and complete vagotomy there is profound decrease in acid secretion in response to a Liebig's extract test meal which is greater than after partial gastrectomy alone. In 51 experiments on four dogs, 17 or 33 per cent were characterized by anacidity.

2. Histamine caused a marked secretion of acid.

3. The intestinal phase of acid secretion was very marked and unusually prolonged.

4. Following operation the gastric samples contained large amounts of a very viscid mucus. When anacidity was present the amount of mucus was very great but it was usually not in evidence when large amounts of acid were being secreted in response to histamine and the intestinal phase. This suggests that the mucus is the result and not the cause of the lowered acidity.

5. The average acidity of the gastric contents of each of the four dogs was definitely below the threshold value of acid necessary for the digestion of living tissues as determined by Dragstedt.

REFERENCES

- (1) WILHELMJ, C. M., F. T. O'BRIEN AND F. C. HILL. *This Journal* 116: 685, 1936.
- (2) WILHELMJ, C. M., F. T. O'BRIEN AND F. C. HILL. *This Journal* 115: 5, 1936.
- (3) WILHELMJ, C. M., H. H. MCCARTHY AND F. C. HILL. Unpublished.
- (4) PRIESTLY, J. T. AND F. C. MANN. *Arch. Surg.* 25: 395, 1932.
- (5) DRAGSTEDT, L. R. *Ann. Surg.* 102: 563, 1935.
- (6) SHAPIRO, P. E. AND B. N. BERG. *Arch. Surg.* 28: 160, 1934.
- (7) HARTZEL, J. B. *This Journal* 91: 161, 1929.

THE EFFECT OF ACETYLCHOLINE AND OTHER CONSTITUENTS OF THE SUPRARENAL GLAND UPON BLOOD SUGAR AND AMINO ACIDS

BURT LINCOLN DAVIS, JR. AND J. MURRAY LUCK

From the Department of Anatomy and the Biochemical Laboratory, Stanford University

Received for publication July 14, 1936

The lowering of the amino acid content of blood in response to injected insulin (1) has been shown by Davis and Van Winkle (2) to be directly due to epinephrine secreted by the suprarenal medulla during insulin stimulation. The possibility that other constituents of the suprarenal gland might be involved in this phenomenon has now led us to investigate acetylcholine, ascorbic acid, and cortin—all of great physiological importance.

Of these substances it appeared most likely that acetylcholine would be active with respect to the blood amino acids. Since the work of Feldberg and co-workers (3) strongly suggests that epinephrine secretion results from a local action of acetylcholine, liberated at the nerve endings in the suprarenal medulla, it seemed possible that the injection of acetylcholine under appropriate conditions would bring about an epinephrine hypoaminoacidemia (cf. also Siehe, 4). Incidentally, the concentration of acetylcholine in the suprarenal medulla has been shown to be relatively high, as great as 0.45 mgm. per kgm. of medullary tissue; its concentration in the suprarenal cortex is much lower (5).

In addition to the amino nitrogen studies analysis of the blood with respect to reducing sugar was indicated, especially since the anticipated discharge of epinephrine might be of such a magnitude as to induce hyperglycemia. It should be noted that several investigators have studied the glycemic behavior of acetylcholine but have reported conflicting results. Bornstein and Vogel (6) and Seo (7) observed a hyperglycemia with acetylcholine, whereas Labbe *et al.* (8), Bruhn and Himwich (9), and Hrubetz (10) noted a diminution in blood-sugar content. Lang and Rigo (11) found that small doses produced hypoglycemia while larger doses were followed by hyperglycemia. The present investigation, we feel, throws further light on the glycemic behavior of acetylcholine and reveals the factors responsible for acetylcholine hyperglycemia.

We may state in advance that, according to our experience, acetylcholine tends to elevate the blood amino acids rather than to decrease

their concentration and that hyperglycemia from administered acetylcholine is only obtained if convulsions intervene. Neither hyperglycemia nor hyperaminoacidemia is dependent upon a discharge of epinephrine since acetylcholine produces a like response in the animal after destruction of the suprarenal medulla. Cortin and ascorbic acid were found to be inactive with respect to blood sugar and amino acids.

EXPERIMENTAL. *Animals.* Adult rabbits, chiefly female, weighing

TABLE 1

Tests of suprarenal medullary function in the operated animals with subcutaneous injection of insulin

ANIMAL NUMBER	WEIGHT	ABSOLUTE CONCENTRATION						PERCENTAGE VALUES					
		Blood sugar			Blood amino nitrogen			Blood sugar			Blood amino nitrogen		
		0 hr.	1½ hrs.	3 hrs.	0 hr.	1½ hrs.	3 hrs.	0 hr.	1½ hrs.	3 hrs.	0 hr.	1½ hrs.	3 hrs.
DOSE: 0.25 U./kgm.—Preliminary test													
	kgm.												
1	4.4				10.6	10.6	10.0				100	100	94
2	3.9				11.5	10.8	11				100	94	95
3	4.1				10.5	10	9.3				100	95	90X
4	2.5				13.1	12.3	13.0				100	94	100
5	2.2	130	40	C	9.1	9.5	8.9	100	31	C	100	104	98
6	1.9	113	40	C	10.2	9.9	9.6	100	35	C	100	97	84
7	2.2	130	66	C	9.2	9.1	8.5	100	51	C	100	98	92
8	2.7	108	51	C	9.5	9.8	9.5	100	47	C	100	103	100
9	4.0	112	68	102	11.1	10.3	9.5	100	61	91	100	92	86X
10	4.6	104	49	69	10.0	9.4	9.5	100	47	66	100	94	95
11	2.4	100	66	77	9.9	9.9	9.5	100	66	77	100	100	96
DOSE: 0.5 U./kgm.—Re-check on doubtful animals one month later													
3	4.1	99	37	28	10.1	9.7	8.7	100	38	28	100	96	86X
9	4.4	97	54	57	10.8	10.8	9.1	100	55	58	100	100	84X
7	3.6	90	52	57	10.7	10.8	10.5	100	58	64	100	101	98

C—Convulsion after 1½ hour sample—glucose administered and 3 hour sugar sample not significant.

X—Animal rejected.

from 2 to 4 kgm., were used. In a number of the animals the suprarenal medulla was destroyed by electric cautery. Histological studies on the suprarenals of such animals revealed an almost complete absence of suprarenal medullary tissue. The 11 animals demedullated by this procedure were subjected to functional tests to determine whether cauterization had been complete. Of the 9 animals retained none showed hyperglycemia after profound fright and none showed hypoaminoacidemia upon injec-

tion of insulin. The results of the insulin test, which we regard as conclusive (2), are presented in table 1.

Blood analysis. Samples of 2.5 cc. were removed from the marginal ear vein at intervals of 1, 2, 3 and 5 hours after commencement of an experiment. Pre-injection samples were also drawn for the determination of basal values. Larger blood samples were considered inadvisable because of the tendency of hemorrhage to induce hypoaminoacidemia (1, 12, 13, 14). The animals were fasted for 48 hours before use.

Blood sugar was determined by the micromethod of Folin (15) which proved to be advantageous because of the small sample (0.1 cc.) required for an analysis.

EPINEPHRINE HYDROCHLORIDE

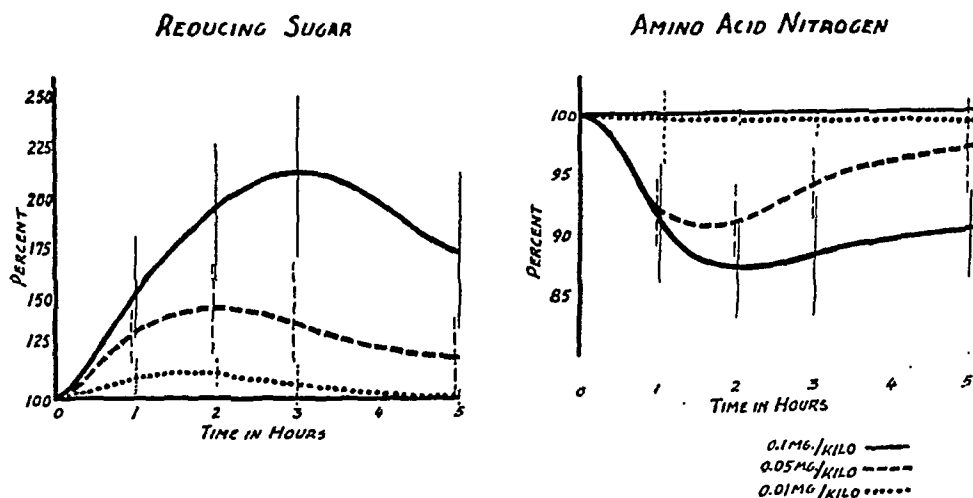


Fig. 1

Amino acid nitrogen was determined on 2 cc. portions by Danielson's modification (16) of Folin's method.

Administered substances. Epinephrine and cortical hormone preparations were obtained from Parke Davis & Co. to whom we are indebted for the gift of these substances. In addition to the crude preparations of the cortical hormone, a highly purified product, Eschatin no. 354, was also used.

Acetylcholine chloride (Hoffman-LaRoche), ascorbic acid (Merck), atropine sulphate (Merck), physostigmine sulphate (Merck), and insulin U 40 (Eli Lilly) were also used.

Experiments with epinephrine. The results of Luck and Morse (12) and Davis and Van Winkle (2) were confirmed, and a quantitative study

was made of the effects of various doses of epinephrine upon the blood sugar and amino acid nitrogen. The purpose of the experiments was to determine the minimum quantities of injected epinephrine necessary to elicit hyperglycemia and hypoaminoacidemia. The commercial preparation of epinephrine hydrochloride (1/1000) was diluted so that the drug was in such a concentration that one cubic centimeter of the solution was administered per kilogram of body weight. In this manner the same amount of diluting saline was administered in all cases, although the quantity of epinephrine varied. To three different groups, composed of at least six animals each, doses of 0.1, 0.05 and 0.01 mgm. of epinephrine per kilogram were injected subcutaneously.

The results of the experiments, recalculated to a common base line, and expressed in terms of percentage change from the pre-injection levels, are presented graphically in figure 1. Each point represents the average value for the group of six or more animals with the average deviation from the mean indicated by a vertical line passing through the point.

The absolute values obtained are indicated by the group averages and average deviations from the means presented in table 2.

The minimal hyperglycemic dose of epinephrine appears to be about 0.01 mgm. per kgm. body weight, a value which is evidently somewhat less than the minimal dose required to elicit hypoaminoacidemia.

In the procedures about to be described acetylcholine was administered in doses of 0.1 and 1.0 mgm. per kilogram of body weight both to normal rabbits and to rabbits in which the suprarenal medulla had been destroyed.

It has been shown by several investigators that acetylcholine is rapidly destroyed in the tissues by choline esterase. Loewi first noted this in his work on the "vagus substance" in 1921 (17). It was again reported by Loewi and Navratil (18), and Englehart and Loewi (19). For this reason acetylcholine must be administered intravenously. Subcutaneous, intramuscular, or intraperitoneal injections have no effect, and it is only by direct introduction into the blood stream, whence it can be rapidly disseminated, that it has any observable action. Even when injected intravenously its activity is of very short duration. Gaddum reports (20) that at 40°C. acetylcholine is almost completely destroyed by human blood in fifteen seconds. The procedure in these experiments was to inject small amounts into the marginal ear vein.

Acetylcholine rapidly deteriorates in solution. The chloride is a very hygroscopic substance, and therefore was kept in a desiccator over phosphorus pentoxide until the time for injections. As they were needed a few crystals were accurately weighed out, dissolved at a concentration of 1 mgm./cc. and injected. The animals convulsed if more than 0.1 mgm. was administered at any one moment. Two-tenths milligram, if given all at once, usually led to the death of the animal in violent convulsions

TABLE 2

Effect of suprarenal gland constituents on reducing sugar and amino acid nitrogen of blood

Group averages and average deviations from the means

SUBSTANCE ADMINISTERED	ANIMALS			REDUCING SUGAR					AMINO ACID NITROGEN					
	Dose, mgm. per kgm. in body weight	Num-ber group	State	Hours after injection					Hours after injection					
				0	1	2	3	5	0	1	2	3	5	
Epinephrine.....	0.1	6	Normal	117±19	179±12	221±21	243±38	198±25	8.4±0.4	7	7.7±0.5	7.4±0.4	7.5±0.5	7.7±0.5
Epinephrine.....	0.05	9	Normal	133±11	174±23	192±32	183±31	145±12	8.2±0.4		7.5±0.3	7.5±0.3	7.7±0.2	7.8±0.5
Epinephrine.....	0.01	6	Normal	108±24	117±22	126±26	116±23	117±13	8.3±0.4		8.2±0.1	8.3±0.4	8.1±0.5	8.2±0.4
Cortical hormone.....	0.5-1.0*	6	Normal	129±7	130±6	125±7	123±4	122±4	7.5±0.2		7.4±0.5	7.4±0.2	7.5±0.2	7.5±0.2
Eseratin.....	0.7*	6	Normal	122±11	122±14	125±12	124±10	128±13	8.8±0.8		8.6±0.8	9.2±0.6	8.9±0.6	8.4±0.6
Ascorbic acid.....	12-25	6	Normal	119±5	117±3	119±4	120±3	117±4	9.8±0.9		9.8±0.7	10.1±0.7	9.8±0.7	9.5±0.7
Acetylcholine.....	1.0	10	Normal	125±11	180±21	169±29	161±29	139±25	8.5±0.4	11.2±0.8	10.5±1.2	9.1±0.7	8.6±0.7	8.7±0.9
Acetylcholine.....	1.0	9	conv.†	121±4	95±13	112±8	123±8	117±7	9.7±0.7		10.6±1.1	9.8±1.0	9.4±0.9	9.4±0.7
Acetylcholine.....	1.0	8	S Ct	119±9	147±10	124±17	118±16	113±12	10.2±0.3		11.8±0.5	10.7±0.3	10.1±0.6	9.7±0.4
Acetylcholine.....	0.1	3	Normal	126±6	121±4	117±3	121±1	119±0	9.2±0.6		9.5±0.3	9.5±0.3	9.4±0.2	9.1±0.2
Acetylcholine.....	0.1	3	N Ct	103±3	114±7	109±4	112±3	109±3	9.9±0.4		9.6±0.1	9.8±0.3	9.5±0.1	9.1±.0
Acetylcholine.....	0.1	7	S Ct	120±4	120±6	117±7	113±16	128±11	9.8±0.8		9.9±0.8	10.0±0.8	9.3±0.6	9.6±0.8
Atropine (2.0 mgm./kgm.) prior to acetylcholine (1.0 mgm./kgm.).....	2.0	6	Normal	116±10	107±15	125±4	132±6	142±25	10.3±0.6	10.3±0.4	10.3±0.5	10.5±0.4	10.3±0.6	10.3±.5
Atropine.....		6	Normal	100±4	103±9	107±10	112±10	120±12	10.3±0.6		10.2±0.6	10.1±0.4	10.2±0.3	10.1±0.4
Physostigmine (0.2 mgm./kgm.) prior to acetylcholine (0.1 mgm./kgm.).....	0.2	1	N Ct	96	113	135	94	100	12.0	11.1	9.7	11.9	12.1	11.9
Physostigmine.....		3	Normal	116±6	125±10	111±3	115±7	122±5	10.2±0.7		9.9±0.6	10.0±0.8	10.1±0.6	9.9±0.6
Insulin (1 unit/kgm.) prior to acetylcholine (1.0 mgm./kgm.).....		3	Normal	132±4	54	49±3	75±13	85±24	11.4±0.4	10.7	9.8±0.2	10.3±0.8	10.0±0.4	10.4±0.4
Insulin (1 unit/kgm.) prior to acetylcholine (1.0 mgm./kgm.).....		2	N Ct	124±1	50±3	46±1	118±31	131	10.2±0.4	9.4±0.2	8.3±0.1	9.7±0.5	8.7	9.5

* Cc. per kgm.

† N C—Normal, convulsed. S C—Suprarenal-demedullated, convulsed.

which simulated the consequence of a profound cranio-sacral stimulation, showing a slow heart rate, profuse salivation and difficult respiration.

A quantity of 0.1 mgm. per rabbit was found to be a convulsive dose for half of the animals. This bore no relationship to the size of the animal, as most of the deaths were observed in those of greater weight. With a dose of 0.1 mgm. those that convulsed were only temporarily so affected. The effects of the drug wore off within a few seconds and after a minute or two another 0.1 mgm. could be given. In order to administer 1 mgm./kgm. it was necessary to give repeated injections of 0.1 mgm. each over the course of a half-hour. Such a dose was administered to a group of twenty rabbits. The time intervals for the subsequent blood samples

ACETYLCHOLINE

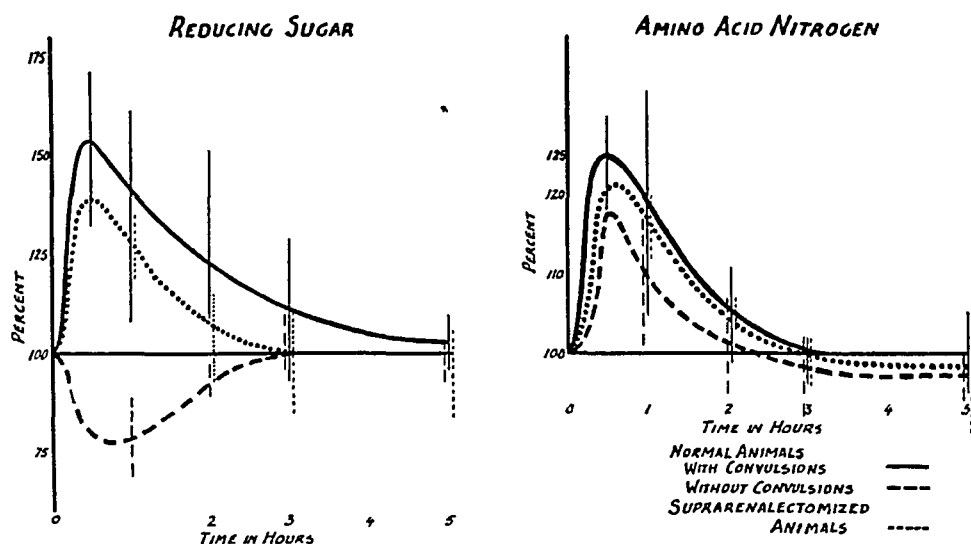


Fig. 2

were computed from the beginning of the injection period, and the thirty-minute sample was, therefore, frequently obtained within a few minutes of the completion of injection when this higher dosage was used. When 0.1 mgm./kgm. was administered there was necessarily a longer interval between the completion of the total dose and the taking of the first sample.

It was soon found that convulsions markedly altered the response to acetylcholine, and the data derived from these procedures have, therefore, been subdivided on this basis. It is interesting to note that animals which had very mild convulsions, sometimes only a few twitches or a single scream, exhibited the same biochemical responses as did those with more marked spasms, and clearly did not fall into the unconvulsed group.

The animals in which the suprarenal medulla had been destroyed were

more sensitive to acetylcholine than normals and convulsions intervened in almost all cases.

The results of the experiments are presented graphically in figure 2 in which group averages are plotted after recalculation to a common base line. Attention is drawn to the intimate relationship which exists between the convulsive state and hyperglycemia, to the hypoglycemia observed in the unconvulsed animals, to the hyperaminoacidemia which acetylcholine always induces and to the non-participation of the suprarenal medulla in respect to the changes both in blood sugar and amino acid nitrogen.

In table 2 the absolute values are expressed in terms of group averages and average deviations from the mean.

Several experiments were next undertaken in which acetylcholine was antagonized by the use of the parasympathetic inhibitor, atropine. Fifteen minutes prior to the use of acetylcholine the animals were injected subcutaneously with atropine sulphate, 2 mgm. per kgm. Such atropinized animals were completely resistant to acetylcholine irrespective of the rate of injection. Convulsions were never observed and, except for a delayed and mild hyperglycemia referable to the atropine alone, there was no effect upon either blood sugar or amino acid nitrogen.

These results are presented in table 2.

We next investigated the possibility of enhancing the action of acetylcholine by inhibition of choline esterase with physostigmine (18, 19). Three control experiments were performed with physostigmine alone. In three others, physostigmine sulphate, 0.2 mgm. per kgm., was injected prior to acetylcholine. It was possible to give only 0.1 mgm./kgm. of acetylcholine and, indeed, this dose induced such profound convulsions in the three rabbits that only one survived. This one animal demonstrated an hyperglycemia of 41 per cent over the preinjection value at the end of the 2-hour interval. The amino acid nitrogen meanwhile dropped to 80 per cent of the basal level. The sugars and amino acid level of the controls remained unaltered. Because of the high mortality accompanying this procedure further experiments were not undertaken, and since there is but one experiment no conclusions can be drawn.

Because of the fact that insulin reduces the amino acid nitrogen (1) and blood sugar of normal rabbits, a study was made of the effect of acetylcholine upon insulinized animals in which the blood had already undergone these changes. One unit of insulin per kilogram of body weight was given subcutaneously after the initial sample had been withdrawn. This was allowed to act for two hours until the insulin effect was at its maximum, and then 1.0 mgm. of acetylcholine per kilogram was given. Of the group of five animals so treated, two underwent convulsions. In these two the blood sugar and amino acid nitrogen rose rapidly.

Indeed, in one, the sugars rose from 38 per cent of the original value to 123 per cent in one hour and simultaneously the amino acid nitrogen rose from 84 per cent to 104 per cent of the preinjection level. The results are presented in figure 3. See also table 2.

The three animals that did not convulse showed no such effect. Again the convulsions appear to be of significance. It would seem as if the acetylcholine were acting here in the same manner as it did in the uninulinized animals and that the effects of these two substances were quite independent.

Experiments with cortical hormone preparations. Two preparations of the cortical hormone were studied. The first was the commercial product

INSULIN-ACETYLCHOLINE

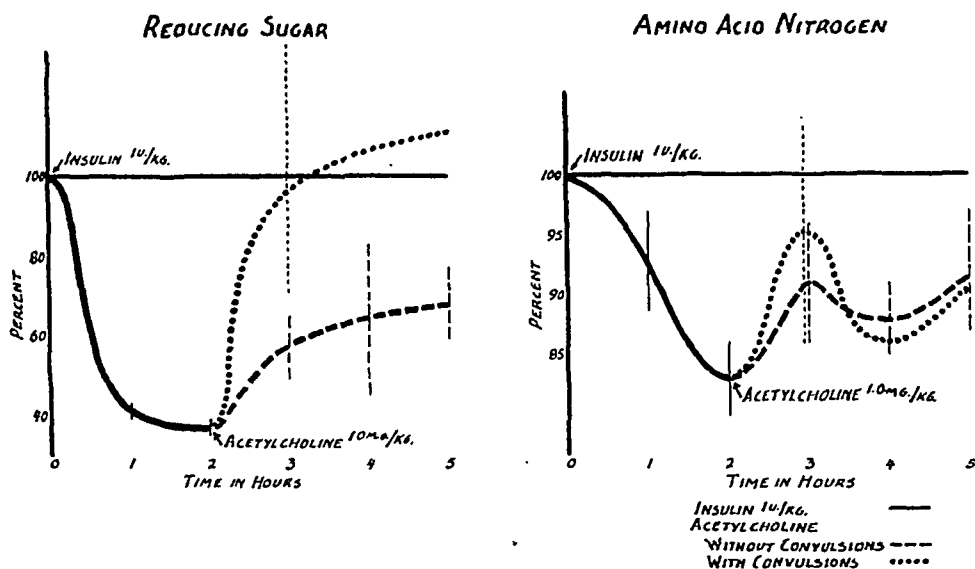


Fig. 3

Eschatin and the second was a more highly purified product known as Eschatin no. 354. These were injected subcutaneously into a group of rabbits in amounts ranging from 0.5 to 1.0 cc. of the extracts per kilogram of body weight. With these doses there were no alterations of the blood sugar or blood amino acid nitrogen levels. The old product Eschatin was administered to two different groups of three animals each, one of which received 0.5 cc./kgm. and the other 1.0 cc./kgm. The newer and more highly purified Eschatin no. 354 was given in a dose of 0.7 cc./kgm. to six rabbits. The data are presented in table 2. On reference to this, the absence of any changes in these blood constituents will be noted. We were quite unable to demonstrate any hyperglycemic effect with cortin, such as Collazo *et al.* (21) and Zunz and La Barre (22) reported; the

minor deviations which we observed all fall within the limits of experimental error.

Experiments with ascorbic acid (vitamin C). Two groups of three rabbits each were injected subcutaneously with 12 and 25 mgm. of ascorbic acid per kilogram of body weight. None of the animals underwent any change in the blood sugar or amino acid nitrogen content (table 2). The fact that these experiments were entirely negative, even with such large amounts of ascorbic acid would make it appear that this substance has no influence upon the blood sugar and amino nitrogen content of normal rabbits. Note that Stepp *et al.* report a marked hypoglycemia in humans after the intravenous injection of 300 mgm. of ascorbic acid (23).

DISCUSSION. The results of most significance are those obtained from acetylcholine, which appears to exert a profound influence on the concentrations of blood sugar and amino acid nitrogen.

The mild hypoglycemia which acetylcholine induces in the unconvulsed animal can be explained most readily either by insulin secretion or inhibition of hepatic glycogenolysis. There is insufficient evidence as yet available to permit one to choose satisfactorily between these possible mechanisms. Experiments upon depancreatized animals are indicated. Hrubetz (10) has made similar observations in rats which gave a mild hypoglycemia in response to acetylcholine.

As for the hyperglycemia observed in certain of the acetylcholine experiments it is clear that epinephrine cannot be regarded as the causal agent. The work of Feldberg *et al.* (3) demonstrates that acetylcholine may be considered as the physiological agent responsible for the transmission of nerve impulses from the fibers of the splanchnic to the cells of the suprarenal medulla with resultant discharge of epinephrine. In our experiments, however, hyperglycemia was consistently observed after destruction of the suprarenal medulla and an epinephrine mechanism could not have been invoked.

We are more disposed to associate the hyperglycemia with convulsions, for elevation of the blood sugar was never observed in the unconvulsed animal but invariably followed upon convulsions, however brief. It seems most probable that the convulsive seizures initiate or accompany a chain of events in the liver which leads to hyperglycemia. The hepatic glycogenolysis, thus postulated, may be of an immediate nervous origin or the result of a humoral mechanism.

A second reason for excluding epinephrine as the hyperglycemic agent is based on the amino nitrogen determinations. It has been previously shown in this laboratory (2) that a discharge of epinephrine induces hypoaminoacidemia. Our present work indicates that the quantity of epinephrine required for this phenomenon is low,—the equivalent of that which would be absorbed from a subcutaneous pocket containing initially 0.01

to 0.05 mgm. of epinephrine per kilogram body weight. In the acetylcholine experiments, far from observing a hypoaminoacidemia, increases in the amino nitrogen content were actually obtained.

Such increases might also be interpreted as hepatic in origin, especially if any weight be given to the fact that hyperaminoacidemia is seldom observed in pathological states except as a consequence of certain severe hepatic lesions. Phenylhydrazine, probably acting as a liver poison, also induces hyperaminoacidemia (24). The immediate source of the blood amino acids, under physiological conditions, has never been revealed but it is not improbable that the liver is a major source.

Finally, we are tempted to suggest that the homeostatic mechanism responsible for regulation of the blood amino acid level may consist of epinephrine and acetylcholine. We have shown in the present research that acetylcholine induces hyperaminoacidemia while our earlier studies demonstrated the hypoaminoacidemic activity of epinephrine. It is also known that suprarenal infusions of acetylcholine provoke a discharge of epinephrine. To completely prove the reciprocal relationship which is now postulated it would be necessary to demonstrate that an over-discharge of epinephrine would elicit a secretion of acetylcholine and that the quantity of either substance in the general circulation or in certain tissues may be influenced by the amino acid level.

SUMMARY

1. The effect of epinephrine, acetylcholine, cortin, and ascorbic acid upon the blood sugar and amino acid nitrogen of rabbits has been studied.

2. Acetylcholine induces hyperglycemia in rabbits if convulsions intervene. In the absence of convulsions, acetylcholine causes a moderate degree of hypoglycemia.

3. Acetylcholine hyperglycemia is obtained in undiminished degree after destruction of the suprarenal medulla; the hyperglycemia is, therefore, not due to a discharge of epinephrine.

4. Acetylcholine causes hyperaminoacidemia both in normal rabbits and after destruction of the suprarenal medulla; it is independent of the incidence of convulsions.

5. Cortin and ascorbic acid are without effect on either blood sugar or amino acid nitrogen.

6. The minimum hyperglycemic dose of epinephrine is slightly smaller than the minimum dose requisite for the production of hypoaminoacidemia.

REFERENCES

- (1) LUCK, J. M., G. MORRISON AND L. F. WILBUR. *J. Biol. Chem.* **77**: 151, 1928.
DANIELS A. C. AND J. M. LUCK. *J. Biol. Chem.* **91**: 119, 1931.
- (2) DAVIS, B. L., JR. AND W. VAN WINKLE, JR. *J. Biol. Chem.* **104**: 207, 1934.

- (3) FELDBERG, W. AND B. MINZ. Arch. exper. Path. u. Pharmakol. **163**: 66, 1931;
Pflüger's Arch. **233**: 657, 1933.
FELDBERG, W., B. MINZ AND H. TSUDZIMURA. J. Physiol. **81**: 286, 1934.
- (4) SIEHE, H. J. Pflüger's Arch. **234**: 204, 1934.
- (5) FELDBERG, W. AND H. SCHILD. J. Physiol. **81**: 37P, 1934.
- (6) BORNSTEIN, A. AND R. VOGEL. Biochem. Ztschr. **122**: 274, 1921.
- (7) SEO, T. Biochem. Ztschr. **163**: 271, 1925.
- (8) LABBE, M., F. NEPVEUX AND JUSTIN-BESANCON. Compt. rend. soc. biol. **100**:
795, 1929.
- (9) BRUHN, M. J. AND H. E. HIMWICH. Proc. Soc. Exper. Biol. Med. **29**: 234, 1931.
- (10) HRUBETZ, M. C. Proc. Soc. Exper. Biol. Med. **33**: 136, 1935.
- (11) LANG, S. AND L. RIGO. Biochem. Ztschr. **192**: 172, 1928.
- (12) LUCK, J. M. AND S. W. MORSE. Biochem. J. **27**: 1648 (1933).
- (13) BISCHOFF, F. AND M. L. LONG. J. Biol. Chem. **84**: 629, 1929.
- (14) DAVIS, B. L., JR., J. M. LUCK AND A. G. MILLER. Biochem. J. **27**: 1643, 1933.
- (15) FOLIN, O. J. Biol. Chem. **77**: 421, 1928.
- (16) DANIELSON, I. S. J. Biol. Chem. **101**: 505, 1933.
- (17) LOEWI, O. Pflüger's Arch. **189**: 239, 1921.
- (18) LOEWI, O. AND E. NAVRATIL. Pflüger's Arch. **214**: 678, 1926.
- (19) ENGLEHART, E. AND O. LOEWI. Arch. exper. Path. u. Pharmakol. **150**: 1, 1930.
- (20) GADDUM, J. H. Ann. Rev. Biochem. **4**: 317, 1935.
- (21) COLLAZO, J. A., J. PAYAL AND I. TORRES. Arch. med. chirurg-espec. **36**: no. 39,
1933.
- (22) ZUNZ, E. AND J. LABARRE. Compt. rend. soc. biol. **120**: 248, 1935.
- (23) STEPP, W., H. SCHROEDER AND E. ALTENBURGER. Klin. Wchnschr. **14**: 933,
1935.
- (24) LEWIS, H. B. AND S. IZUME. J. Biol. Chem. **71**: 33, 1926-27.

A FURTHER STUDY OF THE RELATION OF THE ADRENAL CORTEX TO VITAMIN C¹

JULIA E. LOCKWOOD, DONALD R. SWAN AND FRANK A. HARTMAN

From the Departments of Physiology, University of Buffalo and The Ohio State University

Received for publication July 15, 1936

In an earlier report (Lockwood and Hartman) it was shown that adrenal cortical extract ameliorates the symptoms in avitaminosis C. The presence of vitamin C in the extract was ruled out by passing the preparation through ethyl ether in which it is insoluble. Grollman and Firor (1934) and Svirbely (1935) were unable to confirm these results. The present work confirms the earlier report and proves that the positive effects were not due to the presence of vitamin C. Three methods have been employed. First, the extract has been tested chemically for the presence of vitamin C. Second, the influence of graded doses of extract on the development of the avitaminosis has been determined because it is known that variations in the subminimal protective dose of vitamin C will affect the symptoms in proportion to the quantity given. Third, an extract of liver prepared by the same method as that used for cortical extract has been tried in avitaminosis C. This served as a check on the specificity of the action of the adrenal cortical extract. Liver is rich in vitamin C, although the content of the adrenals is always higher (Bessey and King, 1933; Svirbely, 1933; Yavorsky, Almaden and King, 1934). If the method used for the preparation of the cortical extract removes and preserves some of the vitamin from the adrenal tissue, the same should hold true for liver extract. The cortin content of the cortical extract was found by determining the minimum dosage required to maintain doubly adrenalectomized guinea pigs, of 300 grams weight, in good health. A control series was run in which twice the maximum amount (0.008 mgm. per cc.) of vitamin C, which could have been present in the extract, was injected daily into guinea pigs on a vitamin C-free diet.

The cortical extract was made by extracting the freshly ground adrenal cortex of beef with 95 per cent ethyl alcohol and passing successively through ethyl ether, 70 per cent alcohol (chilled), ethyl ether and crystallite. The finished extract was made up so that one cubic centimeter contained the product of 20 grams of fresh cortex and represented

¹ Aided by a grant from The Rockefeller Foundation.

the minimum daily maintenance dose of cortin for a 300 gram doubly adrenalectomized guinea pig.

Vitamin C determinations were made in a number of our extracts as a check on the method of preparation. In order to obtain maximum values for vitamin C the extract was treated with hydrogen sulfide, according to Tillmans, Hirsch and Dick. The results are given in table 1. Cortical extracts contain reducing substances other than vitamin C which accounts for the higher values given by the iodine method as compared with the indophenol (2,6-dichlorophenol indophenol) method. The extract used in this study was entirely from one lot (no. 131-4) which according to the iodine reduction method contained less than 0.008 mgm. of vitamin C per cubic centimeter. This amount is insignificant. The liver extract represented the product of 25 grams of fresh beef liver per cubic centimeter and contained 0.033 mgm. of vitamin C per cubic centimeter.

TABLE 1
Vitamin C content of extracts of adrenal cortex and of liver

EXTRACT NUMBER	DESCRIPTION	METHOD	VITAMIN C
			<i>mgm. per cc.</i>
136	Extracted with ether once	Indophenol	0.0282
139-41	Extracted with ether once	Indophenol	0.0080
160 S	Extracted with ether twice	Indophenol	0.0296
Ex 1	Purified	Indophenol	0.0034
Liver	Extracted with ether twice	Indophenol	0.0015
136	Extracted with ether once	Iodine	0.103
131-4	Extracted with ether twice	Iodine	0.008
Ex 1	Purified	Iodine	0.0528
Liver	Extracted with ether twice	Iodine	0.033

The spectrographic method was tried but proved less sensitive than the chemical methods for the determination of vitamin C because there was present in the extract an unknown substance whose absorption was near enough to interfere with that for vitamin C.

The experimental procedure followed closely that described by Sherman, LaMer and Campbell (1922). The guinea pigs were obtained from the same source and matched for weight and sex as in the earlier paper of Lockwood and Hartman. The experimental groups are as follows:

1. Positive control group receiving the basal vitamin C-free diet and 3 cc. of orange juice daily.
2. Cortical extract groups receiving the basal diet and two daily intraperitoneal injections of cortical extract ($\frac{1}{2}$ cc. each), a total of 1 cc. daily.
3. A group similar to 2, but receiving twice the amount of cortical extract, i.e., 2 cc. daily.
4. Another group similar to 2, except that the dose of cortical extract was re-

duced to the equivalent of 0.25 cc. daily, but given in a quantity of saline to equal in volume that of group 2, i.e., 1 cc. daily.

5. Liver extract group receiving the basal diet and the intraperitoneal injection of liver extract, $\frac{1}{2}$ cc. twice daily; total 1 cc. per day.

6. Negative control group receiving the basal or vitamin C-free diet and daily intraperitoneal injection of 1 cc. of physiological salt solution.

7. Positive control group treated identically as group 1, but of lower average initial weight.

8. Vitamin C group, similar in weight to 7, receiving the basal diet and intraperitoneal injections of 0.016 mgm. of vitamin C (Merck) in physiological saline per day.

Groups 7 and 8 (broken lines, fig. 1) are not directly comparable with the other groups since the average initial weight of each is definitely

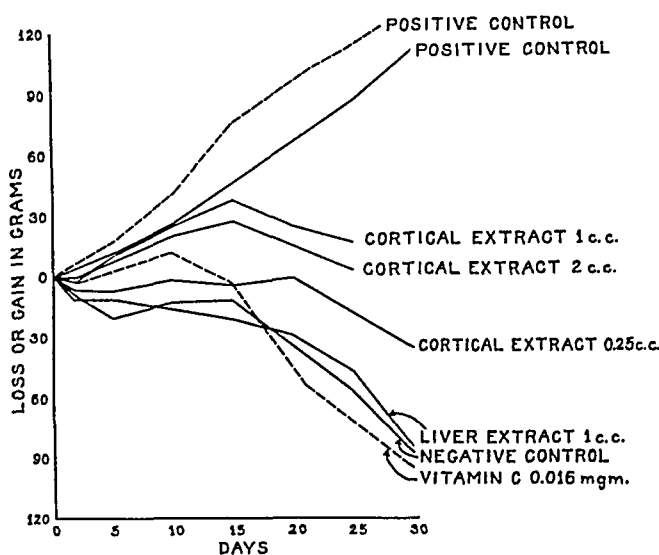


Fig. 1

lower, but they are comparable to each other and are the same as the above groups in all other respects.

Table 2 is a summary of the results obtained and figure 1 shows the average weight curves for each group.

As shown in figure 1, the maximum effect of cortical extract in retarding the development of vitamin C deficiency symptoms was obtained by the administration of 1 cc. It is interesting to note that this is the amount of extract that protected doubly adrenalectomized guinea pigs from developing symptoms of adrenal insufficiency. Two cubic centimeters of the same extract did not give greater protection. The lower average weight curve of these animals may be explained by the fact that the extract was slightly toxic and when administered in larger quantity proved irritating, with a tendency toward congestion at the site of the injection.

Even the 0.25 cc. daily injection of cortical extract gave a weight curve and scurvy score which indicated significant improvement over the negative controls. If the effects noted here, as the result of the use of adrenal cortical extract, were due to the presence of the vitamin, one would expect a graded response in protection in accordance with the quantity of extract given, since in every group receiving cortical extract the vitamin supply is less than a protective dose as the weight curves and scurvy scores indicate. The response is not in proportion to the extract used, which would seem to indicate that the active substance was not vitamin C. These results further show that a maximum influence of the cortical extract on the vitamin C deficiency condition is obtained with daily quantities of 1 cc. This maximum is far short of complete protection. If

TABLE 2

Influence of liver extract, vitamin C and graded doses of cortical extract on the development of scurvy

GROUP	NUMBER OF ANIMALS	EXPERI- MENTAL PERIOD	AVERAGE INITIAL WEIGHT	AVERAGE FINAL WEIGHT	SCURVY SCORE
		<i>days</i>	<i>grams</i>	<i>grams</i>	
1. Positive controls.....	10	30	332	445	0
2. Cortical extract, 1 cc.....	10	25	344	362	3
3. Cortical extract, 2 cc.....	9	25	341	346	3
4. Cortical extract, $\frac{1}{4}$ cc.....	7	30	343	308	6
5. Liver extract, 1 cc.....	9	30	332	268*	12
6. Negative controls.....	9	30	346	301†	17
7. Positive controls.....	8	28	310	441	0
8. Vitamin C (Merek), 0.016 mgm.....	9	28	312	213‡	14

* Three animals dead.

† Four animals dead.

‡ Five animals dead.

vitamin C were the active constituent it should be possible to protect completely from avitaminosis C if a sufficient amount of extract were used.

As shown by the weight curve, the animals receiving liver extract are not measurably different from the negative control group. However, the scurvy score of the liver extract group is midway between the negative control and the 0.25 cc. cortical extract groups and is not far from that for 0.016 mgm. vitamin C. In each case the protective effect is so slight that it cannot definitely be interpreted as positive.

DISCUSSION. Since the adrenal cortex is rich in vitamin C special precautions must be taken to avoid inclusion of the latter in an extract. This has been done in our work by two extractions with small volumes of ether at different stages in the process. The same method was used in the previous work. In order to make certain that this method leaves

the vitamin behind we have tested for its presence in two different ways, viz., by the iodine reduction method and by the indophenol reduction method and find that the amount present is insignificant. The same method of extraction applied to beef liver which, as already related, is rich in vitamin C (contains somewhat less than half the amount of that in the adrenal) (Svirbely, 1933) yielded an extract which contained no significant amount of the vitamin, as shown by the 2,6-dichlorophenol indophenol test and by animal assay. Therefore, the conclusion is justified that this method is capable of removing vitamin C and that the extract which we employed contained negligible quantities of this substance.

Dann and Cowgill (1935) have studied the vitamin C requirement of the guinea pig and concluded that 1 cc. of lemon juice per 100 grams of weight protects the animal from scurvy as determined by the Key and Elphick scale. Bezssonoff has found that 3 cc. of lemon juice daily is the minimum protective dose for a 400 gram animal. If lemon juice contains 0.6 to 0.7 mgm. of vitamin C per cc. (Harris and Ray, 1933) and we use Bezssonoff's value, then the daily protective dose of vitamin C for each guinea pig would be 1.8 to 2.1 mgm. Hou has found that injected vitamin C is twice as effective as that given by mouth. Therefore the daily protective dose by injection would be 0.9 to 1.05 mgm. The amount present in our extract is obviously insignificant.

Consideration of the question of the possible presence of vitamin C in the extract used in the first report is incomplete without comment on the work of Grollman and Firor (1934). They found that 0.25 mgm. of ascorbic acid intraperitoneally injected was definitely more effective in protecting an animal against scurvy, than the same quantity when fed. They concluded that "this is essentially the same result obtained by Lockwood and Hartman in their study of the comparative effects of the oral and intraperitoneal administration of cortical extract and we must conclude that their results are due to the relative efficacy of different modes of administration. Their extracts apparently contained about $\frac{1}{4}$ mgm. of ascorbic acid in 2 cc. (their dosage) which, as we have seen, is not unexpected." This interpretation of results based only on circumstantial evidence is not justified. Because they were unable to obtain positive results with their cortical extract they assume that our extracts must contain vitamin C. An essential difference between the extracts prepared in the two laboratories should be pointed out as it may have a significant bearing on the results. In the preparation of the extract of Grollman and Firor (1933), frozen adrenal tissue is extracted successively with acetone and benzene; the benzene solution is extracted with dilute alkali and then acid and the benzene removed *in vacuo* and finally the residue taken up in physiological saline.

We have found repeatedly that both acetone and benzene are poor

solvents for cortin. Moreover cortin is easily destroyed by dilute alkali. Although they injected the extract from 100 grams of adrenal tissue daily their only statement indicating its cortin content is "This amount of extract corresponds roughly to several times that elaborated by the adrenal glands of the normal guinea pig, as judged from its life-prolonging action in adrenalectomized animals." They give no actual assay value. It is important that the cat, guinea pig or dog be used for this purpose as the rat test is not so conclusive.

Svirbely has recently studied the effects of adrenal cortical extracts on avitaminosis C. He found that ethyl ether does not dissolve the vitamin in significant amounts. The extract which he used was furnished by Dr. E. C. Kendall. Although assayed, its potency is merely stated, "The amount injected in the normal guinea pig should have been adequate (based on assay) to prevent suprarenal deficiency in adrenalectomized animals." He used only 0.2 cc. per day per animal. The dose may have been too small to produce the marked effects which we obtained.

The present work confirms our earlier report that either cortin or some unknown substance from the adrenal cortex delays the onset of scurvy.

SUMMARY AND CONCLUSION

An extract of the adrenal cortex relatively free from vitamin C has been prepared. This extract ameliorates the symptoms of avitaminosis C. An extract of the liver prepared in the same way offers no protection in this avitaminosis. There is an amount of cortical extract which furnishes optimum protection. Greater amounts fail to increase it although the protection is far from complete. Therefore the substance cannot be vitamin C. Cortin or some unknown substance from the adrenal cortex delays the onset of scurvy.

REFERENCES

- BESSEY, O. A. AND C. G. KING. *J. Biol. Chem.* **103**: 687, 1933.
 BEZSSONOFF, N. *Compt. rend.* **183**: 1309, 1926.
 DANN, M. AND G. R. COWGILL. *J. Nutrition* **9**: 507, 1935.
 GLICK, D. AND G. R. BISKIND. *J. Biol. Chem.* **110**: 1, 1935.
 GROLLMAN, A. AND W. M. FIROR. *J. Biol. Chem.* **100**: 429, 1933.
 J. Nutrition **8**: 569, 1934.
 HARRIS, L. J. AND S. N. RAY. *Biochem. J.* **27**: 2016, 1933.
 HOU, H. C. *Proc. Soc. Exper. Biol. and Med.* **32**: 1391, 1935.
 KEY, K. M. AND G. K. ELPHICK. *Biochem. J.* **25**: 888, 1931.
 LOCKWOOD, J. E. AND F. A. HARTMAN. *Endocrinol.* **17**: 501, 1933.
 SHERMAN, H. C., V. K. LAMER AND H. L. CAMPBELL. *J. Am. Chem. Soc.* **44**: 165, 1922.
 SVIRBELY, J. L. *Biochem. J.* **27**: 960, 1933.
 J. Biol. Chem. **111**: 147, 1935.
 TILLMANS, J., P. HIRSCH AND H. DICK. *Ztschr. Untersuch. Lebensm.* **63**: 267, 1932.
 YAVORSKY, M., P. ALMADEN AND C. G. KING. *J. Biol. Chem.* **106**: 525, 1934.

THE EFFECT OF OCCLUSION OF THE OUTFLOW OF PROSTATIC SECRETION ON THE PROSTATE GLAND

JAMES I. FARRELL AND YALE LYMAN

From the Departments of Physiology and Urology, Northwestern University Medical School, Chicago, Illinois

Received for publication July 16, 1936

The effect of occlusion of the outflow of prostatic secretion on the prostate gland has not been studied as far as we have been able to ascertain. The occlusion of the ducts of other external secreting glands such as the salivary glands, the pancreas and liver, as is well known, causes degenerative or atrophic changes in the external secreting cells. Although we anticipated a similar change in the case of the prostate, we thought that hypertrophy might conceivably result. One could not even predict that atrophy would result, because the continuous secretion of the prostate is relatively small. Further, we were interested in ascertaining whether corpora amylacea might be produced under conditions of obstruction of the outflow of prostatic secretion.

METHODS. Three male dogs about four years old were operated on by the following procedure: an incision was made suprapubically to expose the bladder neck and prostate gland. The posterior urethra was grasped between clamps and divided. The cut ends were closed by a catgut suture. Since the prostate in the dog can easily be separated from the vesical neck, this was done, and the cut edge of the bladder closed, and the cut end of the urethra sutured. Care was taken to interfere as little as possible with the blood supply of the gland. A suprapubic cystotomy was done to allow the urine to drain externally (fig. 1).

The circumference of the gland in each dog was measured by placing a string around the gland at its greatest circumference. The dogs were then allowed to survive for a period of five months.

The glands were again measured at autopsy. In addition, histological sections were made of the three glands, and also of normal glands for comparison.

RESULTS. That the operation per se does not lead to significant atrophy was observed in a previous study (1) by making a fistula of the urethra through which the prostatic secretion could drain. In such animals the prostate continues to secrete indefinitely, i.e., at least for seven months.

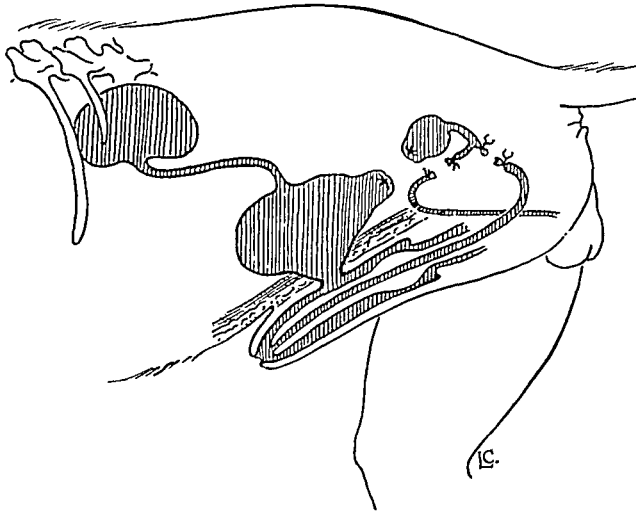


Fig. 1. This diagram shows the method employed for obstructing the secretion of the prostate.

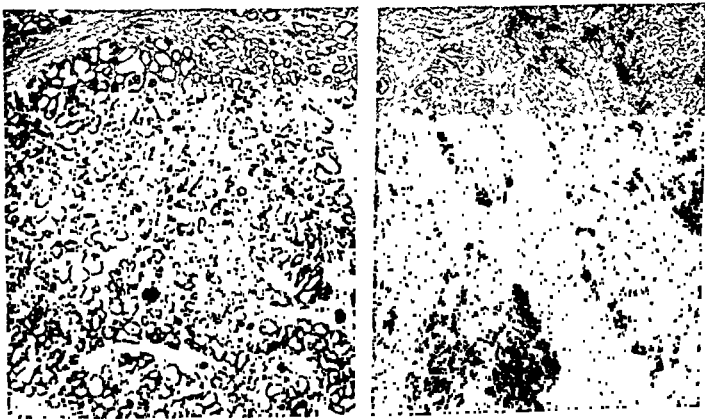


Fig. 2. The section on the left is from a normal prostate; that on the right is from the obstructed prostate of dog 2. The atrophy of the secretory cells is very evident.

TABLE 1
Changes in the circumference of the prostate

DOG	BEFORE	AFTER
1	4½ inches (11.5 cm.)	3 inches (7.6 cm.)
2	5 inches (12.8 cm.)	2¾ inches (6.9 cm.)
3	5.2 inches (13.1 cm.)	3 inches (7.6 cm.)

In each of the three dogs after five months of obstruction, the external secreting cells were found to be degenerated (see fig. 2), but not completely; an apparent or a relative (not a real) increase in gland stroma occurred. The greatest external circumference of the gland decreased (table 1).

CONCLUSIONS

Obstruction to the outflow of secretion from the prostate gland by means of ligating the urethra causes an atrophy of the external secretory cells. Neither hypertrophy nor corpora amylacea were observed. The prostate gland reacts to obstruction to the outflow of its external secretion as do other externally secreting glands.

REFERENCE

FARRELL, J. I. Trans. Am. Genito-Urinary Surgeons 24: 221, 1931.

THE RÔLE OF THE ANTERIOR HYPOTHALAMUS IN TEMPERATURE REGULATION

R. S. TEAGUE AND S. W. RANSON

From the Institute of Neurology, Northwestern University Medical School

Received for publication July 16, 1936

It is now known that the hypothalamus serves as a thermostat regulating body temperature (Isenschmid and Schnitzler, 1914; Keller and Hare, 1932a, b; Bazett, Alpers and Erb, 1933; Ranson and Ingram, 1935; Davison and Selby, 1935). It remains, however, to determine more accurately what part of the hypothalamus serves this function and whether or not one and the same hypothalamic mechanism protects the animal against both rises and falls in external temperature.

METHODS. Adult cats and some that were about two-thirds grown, in good health and free from infections were selected. Lesions were placed with the Horsley-Clarke apparatus in the anterior part of the hypothalamus by Dr. W. R. Ingram for the purpose of producing diabetes insipidus as in earlier experiments reported by Fisher, Ingram and Ranson (1935).

Records of rectal and room temperatures were made daily after the operation for two weeks or until the temperature, if abnormal, had become normal again. Observations were made and duly recorded as to the condition of the cat and whether or not there was any evidence of infection.

In order to determine the ability of the animals to regulate against changes in environmental temperature, cold box and hot box tests were devised. Both normal and operated cats were always tested under the same conditions.

The temperature in the cold box ranged from 30 to 42°F. and the cats as a rule remained in the box for three hours. The hot box (30 x 24 x 12 inches) was regulated as nearly as possible at 102 to 104°F. and was equipped with wet and dry bulbs for humidity determinations. A small stream of air accurately controlled as to volume was passed through the box to keep the humidity from being raised too high by evaporation from the animal's lungs. For the tests in the hot box, the animals were restrained in a comfortable hammock made of canvas stretched on a frame that raised the hammock five inches above the floor of the box. The cats were kept under observation for many weeks and were finally sacri-

ficed to determine the location of the lesion by microscopic examination of the brain.

Rectal temperature readings on the days immediately following the operation. The cats were kept in an incubator regulated at 84 to 86° during the first day or two and sometimes longer as indicated by the stars following the rectal readings in table 1. Even under these conditions five showed slightly subnormal temperatures on the morning after the operation. Each of these five was somnolent the first morning due to prolonged action of the nembutal or to the effect of the lesion in the hypothalamus.

TABLE 1

Morning rectal temperature (F.) on the first eight postoperative days

CAT NUMBER	1	2	3	4	5	6	7	8
20	104.5*	105.6	104.6	103.7	103.2		102.0	102.8
21	97.0*	99.3*	101.3*	98.8		99.3	99.6	100.2
22	101.2*†	103.6*	102.5*	102.7	101.5	102.7	101.0	102.8
23	103.0*†	105.0*	103.2	102.6	101.5	101.2	101.8	100.4
24	102.2*	102.2*	103.2	102.9	101.3	99.6	99.3	100.0
26	87.8*†	100.5*	101.1*	104.6*	101.0*	102.1*	103.6*	102.8*
27	104.4*	103.4*	103.1	104.2	101.5	100.5		101.2
28	104.0*	105.1*	104.3	103.2	103.2	101.5	102.6	101.6
29	94.8*†	104.5*	102.1	101.7	102.3	101.0	98.7	100.1
30	103.9*†	104.2*	103.7*	103.3	105.2	102.6	103.9	103.2
31	102.8*	104.3*	104.0*	103.7*	101.6*	103.4*	101.6*	
32	102.6*	102.8*	102.7*	102.4*			102.0*	101.8*
33	95.0*†	95.3*	102.3*	103.7*	104.1*	104.2*	104.3*	102.9*
34	103.9*	99.9*	100.0*	98.0	97.9	96.8	98.3	99.0
35	105.0*	104.6*	103.5*	105.6	104.7	102.6	103.7	100.8
36	106.1*	103.7*	104.3*	104.7*		107.1*	105.7	103.8
37		104.5*	105.2*	101.7	100.5	100.6	101.7	100.8
38	99.3*†	104.1*	103.5*	103.4*	102.0	101.2	101.9	101.1

* Indicates cat was in the incubator (84-86°F.).

† Indicates cat was still somnolent when temperature was taken.

Cat 33 had an infected ear and cat 38 a respiratory infection.

None was somnolent on the second postoperative day. On the third, fourth or fifth day most of the animals were removed from the incubator without developing subnormal temperatures. Only one cat, 34, ran a temperature much below 99°. Cats and monkeys in which lesions had been placed in the posterior part of the hypothalamus have in our experience shown very much greater loss in the capacity to maintain body temperature up to the normal level.

If 101° or 102° be taken as the normal rectal temperature for the cat it will be seen that a number of the operated animals ran temperatures

distinctly above normal in the incubator and in some cases several days after the operation when they were being kept under ordinary room conditions. It would appear that insofar as these results show a disturbance in temperature regulation there was more often a loss of capacity to keep the temperature down to normal than up to normal. One of the animals (33) had an ear infection and another (38) had snuffles but none of the others had any infection to explain the high temperature.

Results of tests in the cold box. From observations on twenty-nine nor-

TABLE 2
Tests in hot box—Normal cats

CAT NUMBER	TEMPERATURE WHEN PANTING BEGAN	RISE BEFORE PANTING BEGAN	TIME BEFORE PANTING BEGAN
	°F.	°F.	minutes
1	103.7	2.1	32
2	103.6	0.9	20
3	104.2	2.0	40
4	103.2	0.5	30
5	103.4	1.0	30
6	102.0	0.4	8
7	103.7	1.1	20
8	104.3	1.3	27
9	104.6	0.8	11
10	102.5	0.7	14
11	104.2	1.7	53
12	103.0	1.2	29
13	102.3	0.8	12
14	102.8	0.5	27
15	102.4	0.8	10
16	102.9	1.8	50
17	103.4	2.4	45
18	104.0	1.9	93
19	103.6	2.6	148 *
20	103.7	2.6	30
Average.....	103.4	1.4	36

mal cats, including one castrate, subjected to cold air ranging from 30 to 42° for a period of three hours, it was seen that the rectal temperature rose slightly, remained the same, or fell slightly. The highest rise observed was 1.7° and the greatest fall was 0.9°. In no case was the temperature at the end of the period below 100°.

Of thirty-seven operated cats tested as to their ability to maintain normal temperatures against exposure to cold, twenty-six maintained their temperature within the normal range, eleven showed deviations from the normal. In these eleven the rectal temperature in four fell to a

point between 99° and 100°, in three others to a point between 98° and 99° and in only four did it fall below 98°, reaching 96.9°, 96.3°, 95.6° and 94.0° respectively. Shivering was seen in these operated animals as regularly as in the normal controls. It developed in those whose temperature fell below normal as well as in those in which no significant fall occurred. These cold box tests were made several days or weeks after the operation and it is possible that had the tests been made one or two days following the operation more severe temperature disturbances would have been noted.

Results of tests in the hot box. The experiments with normal cats showed a great deal of variation among different cats and in different trials on the same cat as to rise of rectal temperature before panting set in and the time required for this response.

The response of normal cats was as follows: Immediately after the cat was put into the box, the rectal temperature began to rise (unless it was already over 102.0° as a result of struggling in the hammock or excitement, in which case it sometimes fell slightly, and a little later began to rise). With the rise in temperature there was always a concomitant increase in the rate of respiration which rose rapidly and steadily until panting appeared, then the rate of panting continued to increase at intervals with the further rise in temperature until a rate of 200 to 300 per minute was obtained with a temperature of 105.0°. The appearance of panting showed marked variations (table 2); the latent period varied from 8 minutes to 148 minutes; the rise in rectal temperature before panting began varied from 0.4° to 2.6°, with an average of 1.4°F.; the temperature at which panting began varied from 102.0° to 104.6° with the average point at 103.4°. It is to be noted that normal cats do not maintain normal temperatures under these external conditions, but physiological mechanisms are invariably called into play to their fullest extent: polypnea, cutaneous vaso-dilatation as seen on the ears and pads of the feet, and sweating on the feet. Salivation was noted along with the polypnea. As the rectal temperature rose the cats became more and more excited; frequent cries, dilated pupils, and struggling were observed with the animals obviously in distress.

The operated cats presented responses to heating which in most cases were abnormal. Panting began at a higher rectal temperature than in normal cats. Many did not pant although their rectal temperature reached the high levels of 105° or 106° (table 3). Some did not even exhibit an increase in respiratory rate at all at these temperatures; in most cases there was no sweating on the pads of the feet; a few of the animals showed a surprising tolerance to the heat and an apparent indifference to the hyperpyrexia, lying still and resting with normal respiratory rate, seemingly very calm and in no distress whatsoever.

The gradient of rise in rectal temperature when this is plotted against the respiratory rate was much steeper in the operated than in the normal animals (fig. 1). The steepness of this gradient is as significant as the

TABLE 3
Tests in hot box—Cats with anterior hypothalamic lesions

CAT NUMBER	DAYS AFTER OPERATION	TEMPERATURE WHEN PANTING BEGAN	RISE BEFORE PANTING BEGAN	TIME BEFORE PANTING BEGAN	TIME IN BOX	TEMPERATURE REACHED WITHOUT PANTING
		[°] F.	[°] F.	minutes		[°] F.
1	36	107.0	4.2	93		
2	36	105.0	4.5	180		
3	29	104.7	4.8	160		
4	27	106.4	6.5	120		
5	28	103.8	2.8	110		
6	32	103.2	2.8	100		
7	28	105.6	4.0	72		
8	20	104.1	2.1	100		
9	20	105.0	3.2	51		
10	20	105.0	3.2	60		
11	33	104.9	3.4	80		
12	20				190	106.4
13	12				210	105.8
14	11				140	106.3
15	10	104.4	4.4	140		
16	9				148	106.2
17	7				26	106.4
18	32	103.4	3.4	84		
19	130	105.3	7.3	238		
20	17	105.3	3.5	122		
21	17	104.3	1.6	64		
22	24	104.5	3.3	134		
23	21	105.9	4.4	100		
25	27	106.0	4.6	127		
26	27				170	103.3
27	14	105.3	4.7	86		
28	11				47	106.3
29	9				102	106.2
31	91				180	104.8
32	29				170	106.3
33	38	105.6	4.2		120	
38	16	105.0	3.5	250		
Average.....		105.0	3.9	117.7	136.6	105.8

temperature at which panting occurred. Thus, cats 5 and 6 which panted at temperatures below 104° gave data which when plotted showed steep gradients similar to those of the other operated cats. Three cats (8, 18

and 21) gave data which when plotted yielded curves more like the normal and these cats also panted at moderately low temperatures. These three cats, therefore, gave fairly normal reactions in the hot box.

It is clear that the capacity to regulate against heat may be greatly impaired without any loss in the ability to regulate against cold. For instance, four cats in which the hot and cold box tests were both made within a few days after the operation reacted normally against cold but failed to pant even when the rectal temperature was raised above 106° (cat 14, cold box test on 9th and hot box test on the 11th day; cat 16, cold box test on the 7th and hot box test on the 9th day; cat 17, cold

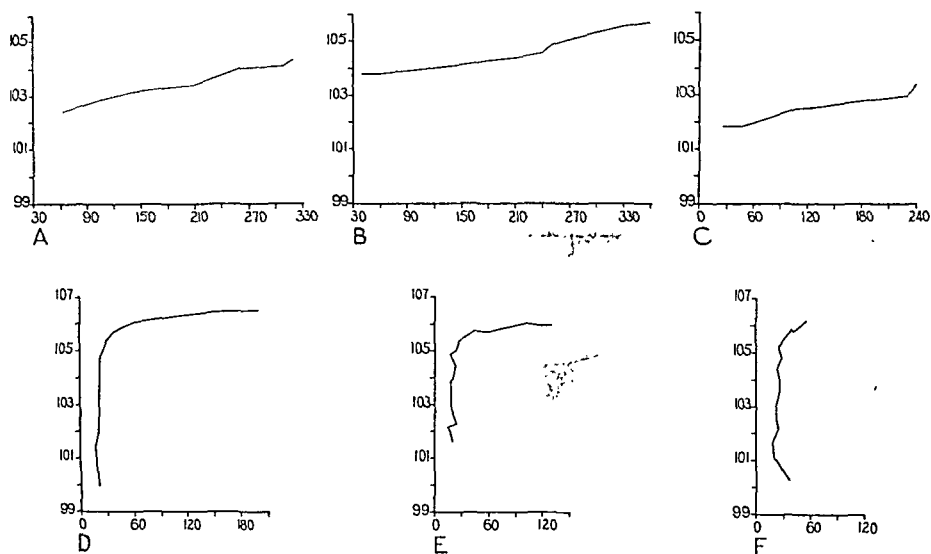


Fig. 1. Graphs showing rise in rectal temperature in degrees F. plotted against the number of respirations per minute and illustrating the effect of heating the animals in a box maintained between 102 and 104°F . A, B and C, three normal cats; D, E and F, three cats with lesions in the anterior hypothalamus; D, cat 4; E, cat 25; F, cat 16.

box test on the 6th and hot box test on the 7th day; cat 28, cold box test on the 9th and hot box test on the 11th day).

As a result of the much greater respiratory rate, the normal cats eliminated more water from their lungs and caused a more rapid rise in the humidity of the air in the box. The average increase in humidity divided by the average number of minutes that the animals were in the box gives a humidity quotient of 0.1275 for the normal cats and 0.057 for the operated series. In other words, the greater increase in respiration in the normal cats resulted in more than twice as rapid an increase in the humidity of the air in the box as occurred when the operated animals were being tested.

Cat 27 may be taken as typical of those which showed a decreased re-

sistance to heat. This animal ran a temperature definitely above normal, varying between 103.1° and 104.4° during the first four postoperative days (table 1). During the first two days it was in the incubator; but normal cats maintain normal temperatures under these conditions. During the third and fourth days the cat was in a room the temperature of which varied between 68° and 70°F . Twelve days after the operation the cat was placed in a cold box (30 to 42°) for two hours and fifty-five minutes and its rectal temperature dropped from 101.3° to 100.9° or only 0.4°F . On the fourteenth postoperative day it was put in the hot box and its temperature reached 105.3° before panting began. The curve of temperature plotted against respiratory rate had a steep gradient.

The lesions in this cat damaged extensively the optic chiasma and the

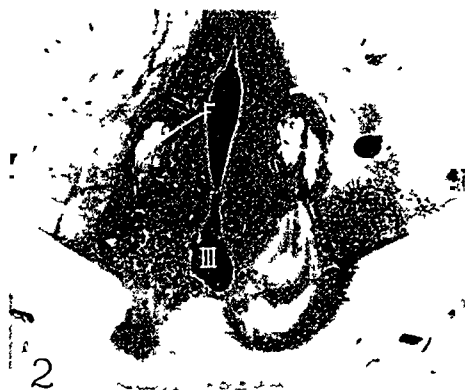


Fig. 2

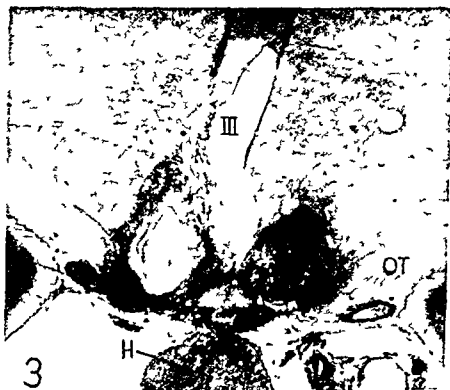


Fig. 3

Fig. 2. Section through the hypothalamus of cat 27 showing involvement of the optic chiasma and the suprachiasmatic region. F, fornix; III, third ventricle. Weil stain.

Fig. 3. Section through the hypothalamus of cat 27 showing destruction of the infundibulum and adjacent part of the floor and walls of the third ventricle. H, anterior lobe of hypophysis; OT, optic tract; III, third ventricle. Cresyl violet

supraoptic portion of the hypothalamus (fig. 2) extending backward through the tuber destroying the infundibulum (fig. 3). The lesions on the left side extended backward almost to the mammillary body but that on the right left much of the ventromedial hypothalamic nucleus intact. The lesions are somewhat larger and extend further dorsally and laterally than in most of the cats of this series. Extensive damage was done to the optic chiasma, supraoptic commissures, anterior hypothalamic area and nucleus and to the dorsomedial and ventromedial hypothalamic nuclei. Some damage was done to the filiform and supraoptic nuclei and the nucleus ovoideus was destroyed on one side.

In all of the animals studied in this investigation the lesions involved the anterior part of the hypothalamus including the floor of the third

ventricle as far back as the infundibulum. In most instances more or less extensive damage was done to the optic chiasma. Exceptions to this rule are furnished by two of the three cats which showed fairly normal reactions in the hot box. In cat 8 the lesion reached forward as far as the posterior border of the optic chiasma on one side only and in cat 21 the lesions were in the tuber and did not even approach the chiasma. The brain of the other cat which behaved normally in the hot box is not available for study.

In those cats which were unable to keep the rectal temperature quite up to normal during exposure to cold the lesions extended farther backward than usual destroying the rostral part of the posterior hypothalamic nucleus and in a few cases also the rostral part of the mammillary bodies. These observations on the cat are in agreement with the results of experiments made on the monkey which have shown that a transient hyperthermia may result from lesions confined to the anterior part of the hypothalamus while bilateral lesions which extend back dorsolateral to the mammillary bodies cause hypothermia (Ranson, Fisher and Ingram, 1937).

The data available are not sufficient to enable one to determine just what structure in the anterior part of the hypothalamus is concerned in holding the body temperature down to normal under conditions which tend to cause a rise. But it seems clear that a mechanism responsible for such regulation exists in this region. Of equal interest is the fact that such extensive damage to the anterior part of the hypothalamus involving the suprachiasmatic region and the floor of the third ventricle back to and including the infundibulum causes no obvious disturbance in the capacity to prevent abnormal falls in body temperature. These results are not in agreement with those of Frazier, Alpers and Lewy (1936).

SUMMARY

Cats with lesions in the anterior part of the hypothalamus ran normal temperatures or in some cases even showed a slight hyperthermia. They showed a diminished capacity to prevent abnormal rises in body temperature and when placed in a box at 102 to 104° they did not react as readily as normal cats by increased respiratory rate and panting. The ability to regulate against cold was not seriously impaired in these animals.

REFERENCES

- BAZETT, H. C., B. J. ALPERS AND W. H. ERB. *Arch. Neurol. and Psychiat.* 30: 728, 1933.
DAVISON, C. AND N. E. SELBY. *Arch. Neurol. and Psychiat.* 33: 570, 1935.
FRAZIER, C. H., B. J. ALPERS AND F. H. LEWY. *Brain* 59: 122, 1936.

- FISHER, C., W. R. INGRAM AND S. W. RANSON. Arch. Neurol. and Psychiat. **34**: 124, 1935.
- ISENSCHMID, R. AND W. SCHNITZLER. Arch. f. exper. Path. u. Pharmacol. **76**: 202, 1914.
- KELLER, A. D. AND W. K. HARE. Proc. Soc. Exper. Biol. and Med. **29**: 1067, 1932a.
Proc. Soc. Exper. Biol. and Med. **29**: 1069, 1932b.
- RANSON, S. W., C. FISHER AND W. R. INGRAM. Arch. Neurol. and Psychiat. In press, 1937.
- RANSON, S. W. AND W. R. INGRAM. Proc. Soc. Exper. Biol. and Med. **32**: 1439, 1935.

EMOTIONAL LEUCOPENIA IN RABBITS¹

L. B. NICE AND H. L. KATZ

From the Department of Physiology, The Ohio State University

Received for publication July 20, 1936

Several recent investigators have shown that emotional states influence the total number of white blood cells in the general circulation. Mora, Amtman and Hoffman (1926) found an emotional leucocytosis ranging from 30 to 150 per cent in dogs and cats. Menkin (1928) observed an emotional mononucleosis in cats. Cheng (1930) and Nye and Barrs (1932) reported that restraint of rabbits for a few hours in the abdomen-up position caused a leucopenia. Cheng (1930) reported this to be a lymphopenia. On the other hand Schweizer (1932) found no apparent relation between the position of a rabbit and the leucocyte count, and Katsura (1930) described a leucocytosis in the blood of rabbits one hour after binding.

In this study we have investigated the number of leucocytes in the blood during emotional states.

PROCEDURE. Rabbits were chiefly used in our experiments, but for comparative purposes a group of cats were also employed. All of our animals were full grown and both sexes were represented.

The blood was obtained from a needle puncture in a marginal ear vessel in the rabbits and from the cats by snipping the margin of an ear with a sharp pair of scissors for each sample. The first drops of blood were always discarded. The ear vessels were slightly dilated by warmth from an electric bulb before securing blood.

Central blood from the rabbits was taken directly from the heart by means of a hypodermic syringe.

All animals were deprived of food and water for 16 to 24 hours previous to an experiment so as to minimize the effects of digestion and absorption on the blood picture.

For obtaining blood for all the counts on peripheral blood in the normal and recovery periods the rabbits and cats were in the quiet upright position. But during excitement they were restrained for about 10 minutes back downwards to an animal holder and excited by being teased with a

¹ A preliminary report was presented to the American Physiological Society March 1934.

weak interrupted faradic current for approximately 3 minutes. All of our animals showed distinct signs of sympathetic stimulation under excitement.

The blood was collected in standardized Trenner automatic pipettes and each sample diluted with a one per cent acetic acid solution which was slightly colored with gentian violet. A uniform procedure was followed in diluting the blood, shaking the pipettes, filling the hemocytometers and

TABLE 1

Protocols. The number of leucocytes in the peripheral blood before, during, and after emotional excitement

SEX	FIRST NORMAL	SECOND NORMAL	EXCITED	FIRST RECOVERY	SECOND RECOVERY
A. Normal rabbits					
F	15,300	15,450	10,200	13,500	15,100
F	17,700	14,500	13,100	18,500	20,000
M	9,800	7,800	6,900	6,200	6,200
M	10,100	11,400	8,300	9,000	8,750
F	8,400	7,600	7,300	7,200	9,500
Average....	12,260	11,350	9,560	10,880	11,910
B. Splenectomized rabbits					
F	11,500	15,500	5,200	12,700	15,000
F	33,200	28,900	12,200	29,500	25,700
M	8,600	9,350	6,200	8,750	9,000
M	14,970	12,500	10,600	14,500	13,000
F	6,600	8,600	4,950	5,950	6,100
Average....	14,974	14,970	9,830	14,280	13,760
C. Normal cats					
F	26,100	28,300	47,000	37,700	34,200
F	18,000	18,100	26,500	21,400	22,000
M	11,100	11,550	14,750	13,900	12,900
M	14,500	14,500	24,000	14,300	14,500
Average....	17,425	18,113	28,063	21,825	20,900

in the counting of the cells in every case. At least 400 cells were enumerated in each test. The values recorded are the averages of the counts from two double chambered Neubauer ruled hemocytometers which were filled from the same diluting pipette.

RESULTS. *Peripheral blood.* A series of five counts at 15 minute intervals, two in the normal state, one in the excited state and two during recovery were made on the peripheral blood in a group of 23 tests on rab-

bits. The average of the counts in the normal quiet state was 10000 per cu. mm. of blood, during excitement 8300, and during recovery 9300. The average decrease during excitement was 17 per cent. In table 1-A protocols from this group of animals are shown.

In a second series of 14 normal rabbits four consecutive counts at 15 minute intervals were made in the normal quiet state, one during excitement and four during recovery. These counts as in the previous group varied in the different individuals, but on the whole they showed a decided leucopenia during excitement. The average of the counts in the quiet state was 11000 and during excitement 7490, a decrease of 31.9 per cent below the normal level. The four recovery counts averaged 10150.

Central blood. In order to compare the effect of excitement on the

TABLE 2

Average changes in the number of leucocytes in the peripheral blood of normal rabbits and cats, the heart blood of normal rabbits and the peripheral blood of splenectomized rabbits

TYPE OF ANIMAL	NUMBER OF TESTS	TOTAL LEUCOCYTES—QUIET STATE	TOTAL LEUCOCYTES DURING EXCITEMENT	PER CENT CHANGE
Normal rabbits (P).....	23	10,000	8,300	-17.0
Normal rabbits (P).....	14	11,000	7,490	-31.9
Normal rabbits (H).....	19	9,000	7,800*	-14.3
Splenectomized rabbits (P).....	17	14,100	7,500	-45.4
Cats (P).....	10	16,100	22,800	+41.6

P = Peripheral blood; H = Heart blood.

* Delayed leucopenia.

number of leucocytes in central blood with that in peripheral blood a series of 20 counts was made on blood obtained from the hearts of rabbits. These averaged 9000 in the quiet state, 9100 immediately after excitement, 7,800 during the first recovery and 8700 during the second recovery. Thus there was no immediate leucopenia, but a delayed one of 14.3 per cent.

Effect of splenectomy. In a series of 16 tests made on splenectomized rabbits the number of white cells in peripheral blood in the normal quiet state averaged 14100, during excitement 7500, while during recovery it was 14035 or back to the normal condition for this group. In these experiments there was a decrease of 45.4 per cent in the number of leucocytes during excitement. The removal of the spleen seems to have intensified the leucopenia. Table 1-B gives protocols from this group.

Cats. In order to find whether the leucopenia observed in rabbits

occurs also in cats under a similar condition of emotional excitement, a series of ten observations was made on the peripheral blood of these animals in the normal, excited, and recovery states. In contrast to the leucopenia found in rabbits each one of these cats showed a decided leucocytosis during emotional excitement. The number of leucocytes averaged 16,100 in the normal quiet state and 22800 during excitement, which is an increase of 41.6 per cent, while during recovery the counts averaged 17125. Protocols are shown in table 1-C.

The five groups. Table 2 gives a summary of the average changes in the number of leucocytes in each group of our animals during excitement. Although there was a decided leucopenia in the peripheral blood of all of our rabbits and a marked leucocytosis in cats under emotional excitement, there was a decided decrease in the number of polymorphonuclear leucocytes with a concomitant increase in the lymphocytes in all of these groups of animals.

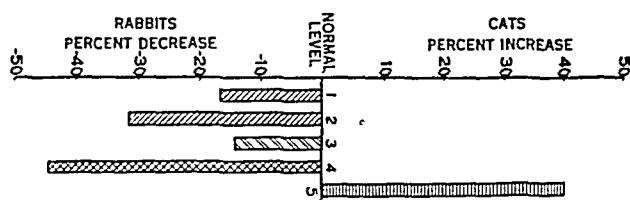


Fig. 1. Summary of percentage changes in the number of leucocytes.

1 = 23 tests on peripheral blood of normal rabbits. 2 = 14 tests on peripheral blood of normal rabbits. 3 = 19 tests on heart blood of normal rabbits. 4 = 17 tests on peripheral blood of splenectomized rabbits. 5 = 10 tests on peripheral blood of normal cats.

Figure 1 shows the average percentage changes in the number of leucocytes from the normal level in each one of our groups of rabbits and cats during emotional excitement.

DISCUSSION. Our average normal leucocyte values of 9000 to 11000 per cu. mm. of blood are within the range reported for rabbits by Pearce and Casey (1930) and Jackson and Stovall (1930). In this investigation we have been concerned with relative changes in the number of white blood cells during quiet and emotional states and not with absolute values.

A considerable amount of evidence indicates that leucocytes may be sequestered in the capillaries or be mobilized into the blood stream during emotional states. (For a review see Garrey and Bryan, 1935.) Wells (1917) reported an increased number of neutrophils in the capillaries of the liver, lungs and spleen during leucopenia caused by injecting dead bacteria into rabbits. Shigeru Momouye (1929) showed histologically that large numbers of leucocytes are immobilized in the capillaries of the lungs, intestine and bone marrow. Nye and Barrs (1932) reported an

abundance of granular leucocytes present in the liver, lungs and to a less extent in the spleen. Seyderhelm and Oestrich (1927) described the filtering and sequestering action of the lungs, liver, and spleen.

In the emotional leucopenia in our rabbits and in the leucocytosis in the cats there was a decided decrease in the percentage of the polymorphonuclear leucocytes in the blood with a concomitant increase in the lymphocytes. This will be described in a forthcoming communication.

Our results with the leucopenia in rabbits agree in part with Cheng (1930) and Nye and Barrs (1932). The leucocytosis found in cats is in agreement with other workers.

SUMMARY

This investigation was undertaken to determine the effect of emotional excitement on the distribution of leucocytes in the blood of rabbits.

In a series of 23 tests on the blood of rabbits in the normal quiet state the total number of white blood cells in peripheral blood averaged 10000 per cu. mm. of blood. During excitement this number decreased to 8300 or 17 per cent, showing a definite leucopenia.

In a series of 14 tests on the peripheral blood of a second group of normal rabbits the average white cell count was 11000 per cu. mm. of blood and during excitement 7490, which is a reduction of 31.9 per cent.

A series of 20 tests on blood taken directly from the heart showed a delayed leucopenia of 14.3 per cent after excitement.

In a series of 16 tests on splenectomized rabbits the number of leucocytes decreased from 14100 in the normal quiet state to 7500, or 45.4 per cent during excitement.

There was a decided leucocytosis during excitement in cats. The total number of leucocytes in the peripheral blood in a series of 10 consecutive tests on cats averaged 16100 per cu. mm. of blood in the quiet state and was augmented to 22800 or 41.6 per cent during excitement.

REFERENCES

- CHENG, D. C. *Am. J. Hyg.* 11: 449, 1930.
EDWARDS, H. T. AND W. B. WOOD. *Arbeitsphysiologie* 6: 73, 1932.
GARREY, W. E. AND W. R. BRYAN. *Physiol. Reviews* 15: 597, 1935.
JACKSON, J. W. AND W. D. STOVALL. *J. Lab. and Clin. Med.* 16: 82, 1930.
KATSURA, S. *Tohoku J. Exper. Med.* 16: 241, 1930.
MENKIN, V. *This Journal* 85: 489, 1928.
MORA, J., L. AMTMAN AND J. HOFFMAN. *J. A. M. A.* 86: 945, 1926.
NYE, R. N. AND V. R. BARRS. *Folia Haemat.* 47: 402, 1932.
NICE, L. B. AND H. L. KATZ. *This Journal* 109: 80, 1934.
PEARCE, L. AND A. E. CASEY. *J. Exper. Med.* 52: 145, 1930.
SCHWEIZER, M. *Quart. J. Exper. Physiol.* 22: 295, 1932.
SEYDERHELM, R. AND E. OESTRICH. *Ztschr. f. d. ges. Exp. Med.* 56: 503, 1927.
SHIGERU, MOMOUYE. *J. Nagasaki. Med. Soc.* 7: no. 1, 1929.
WELLS, C. W. *J. Inf. Dis.* 20: 219, 1917.

THE AMERICAN JOURNAL OF PHYSIOLOGY

VOL. 117

DECEMBER 1, 1936

No. 4

THE RESPIRATORY RESPONSES OF PRE-ADOLESCENT BOYS TO MUSCULAR ACTIVITY¹

EDWARD C. SCHNEIDER AND C. B. CRAMPTON

From the Department of Biology, Wesleyan University, Middletown, Connecticut

Received for publication March 10, 1936

This is a further study of rapidly growing pre-adolescent boys, ages 9 to 13 years, whose cardio-vascular responses we reported in an earlier article. In this paper consideration is given to the respiratory, metabolic, and pulse rate responses to graded intensities of work on the bicycle ergometer, to work pushed to fatigue, and to the recovery processes after strenuous effort.

The boys usually reported for work immediately after the close of the afternoon session of school. It was our custom to have each subject rest quietly in a chair for 15 or 20 minutes, then mount the bicycle and again rest when prepared for the experiment with mouth-piece and nose-clip in place. The control determinations of the metabolic and respiratory factors and of the pulse rate were followed for a period of 10 minutes. The respired air was collected in Douglas bags. In the series of experiments dealing with the effects of graded intensities of work, it was customary to have the subject carry the load for 3 minutes before the collection of expired air was begun. Between periods of work ample time was allowed for complete recovery.

The relation of the oxygen intake to the load of work. With adults who are accustomed to serving as subjects of experimentation the absorption of oxygen generally shows a linear relationship to the intensity of effort. Our data, as summarized in table 1, obtained from the pre-adolescent boys, fail to establish that relationship in them, partly due to an inordinate augmentation in the consumption of oxygen when the lightest load was undertaken. When overloads of work were carried the oxygen consumption of the boys was less than that of young men under the same load. This difference in the two groups gives another reason for failure to establish the linear relationship between oxygen consumption and intensity of effort.

¹ The expense of this investigation has been met by a grant from the Charles Himrod Denison Fund.

We have shown elsewhere (Schneider, 1931) that among young men when an over-load is undertaken the expected increase in oxygen intake often falls short. In only one of the boys was this clearly so; namely, in R. B. (see table 1) who was the weakest of the group. In this case the oxygen intake increased by 243 cc. when the load was stepped up from 1500 to 3000 ft.-lbs., and by 314 cc. when the load was raised from 3000 to 4500 ft.-lbs.; yet, when 6000 ft.-lbs. were carried, the oxygen intake was augmented only 114 cc.

Minute volume and frequency of breathing. The data for the minute volume and frequency of breathing are summarized in table 2. It has been found that the minute volume, frequency, and depth of breathing for men correlate with the amount of physical work accomplished per minute as

TABLE 1

SUBJECT	CUBIC CENTIMETERS OF OXYGEN ABSORBED PER MINUTE DURING WORK					INCREASE IN OXYGEN CONSUMPTION PER INCREASE IN LOAD			
	Rest	1,500 foot-lbs.	3,000 foot-lbs.	4,500 foot-lbs.	6,000 foot-lbs.	1,500 foot-lbs.	3,000 foot-lbs.	4,500 foot-lbs.	6,000 foot-lbs.
R. B.	243	754	997	1,311	1,425	511	243	314	114
J. B.	305	894	1,126	1,510	1,750	589	232	384	240
J. H.	298	936	1,299	1,503	1,785	638	363	204	282
H. C.	297	767	1,071	1,373	1,643	470	304	302	270

TABLE 2

SUBJECT	MINUTE-VOLUME OF BREATHING IN LITERS					FREQUENCY OF BREATHING PER MINUTE				
	Rest	1,500 foot-lbs.	3,000 foot-lbs.	4,500 foot-lbs.	6,000 foot-lbs.	Rest	1,500 foot-lbs.	3,000 foot-lbs.	4,500 foot-lbs.	6,000 foot-lbs.
R. B.	6.8	16.57	22.10	31.03	34.98	13	26	26	33	33
J. B.	9.9	20.32	26.04	37.19	43.67	16	30	37	39	43
J. H.	7.7	21.98	32.76	41.79	51.10	19	33	42	43	43
H. C.	7.8	20.03	25.63	34.30	42.14	13	30	29	32	36

long as the load is moderate. It will be observed that our boys failed to conform to these linear relationships. During rest the volume and frequency of breathing of boys are not noticeably different from those of men. During physical work, however, the boys breathe more frequently but inspire less air per minute than men.

Tidal air. Here again when at rest the boys do not differ materially from young men. For the boys the resting tidal air as they sat on the bicycle ergometer ranged from 403 to 675 cc. During work, however, there is a well defined difference in the depth of breathing of the two ages, which shows up under all loads of work. Thus with a load of 6000 ft.-lbs. the tidal air per breath of the boys ranged between 1032 and 1190 cc., while that of men ranged from 1378 to 2891 cc.

The respiratory dead space. For 3 of the boys during rest the dead space was 128, 152, and 158 cc., respectively. For adults it is said to range between 100 and 175 cc. Henderson finds it is about 150 cc. Normally it increases during physical effort. For our boys the following increases were typical: J. B. at rest 152 cc., with a load of 3000 ft.-lbs. 161 cc. and with a load of 4500 ft.-lbs. 190 cc. The increases in the dead space shown by the boys when at work are somewhat smaller than occur in men when the minute volume of breathing is augmented to the same degree.

The ventilation equivalent for oxygen. This has been defined by Knipping and Moncrieff as the volume of air which has to be inspired for each 100 cc. of oxygen absorbed. They found that its value for normal adults is approximately 2.4 liters and that under experimental conditions it remains unaltered after taking food or moderate exercise. Among our boys when at rest the ventilation equivalent ranged from 2.6 to 3.3 liters, which was somewhat above the normal value found by Knipping and Moncrieff. By light work, 1500 ft.-lbs., the V.E. was lowered, but as the load of work was augmented it rose slightly. As there was no instance of a large increase in the V.E., it may be assumed that in no case, even with a load of 6000 ft.-lbs., was there evidence of a large discharge of lactic acid into the blood stream, in that such a discharge should so stimulate the respiratory center that the volume of breathing would rise without a comparable increase in oxygen consumption.

Respiratory quotient. The R.Q. of rest gives no indication that any boy was overbreathing. There is nothing exceptional or unusual in the exertion records. The R.Q. rises with the metabolic rate, as it does for adults, as shown by Douglas, Haldane, Henderson, and Schneider and other workers.

Alveolar air. Sampling of the alveolar air with the subject at rest was done by the Haldane-Priestley method and during exertion by a modification of the Henderson-Haggard method. All data on alveolar air here recorded are supported by several experiments made under identical conditions. The average resting values for the boys were as follows: CO₂ in mm. Hg. R. B. 40.3, J. B. 38.6, and J. H. 38.8; O₂ in mm. Hg. R. B. 101.2, J. B. 100.5, and J. H. 99.3.

Physical exertion invariably caused the alveolar oxygen pressure to rise. The data for J. B. are the most extensive. His alveolar oxygen with a load of 3000 ft.-lbs. advanced from an average of 100.5 mm. during rest to an average of 104.5 mm.; with a load of 4500 ft.-lbs., to an average of 106.4 mm.; and with a load of 6000 ft.-lbs., to an average of 108.9 mm. For J. H. with a load of 3000 ft.-lbs., the oxygen pressure rose from a resting value of 99.3 mm. to an average of 108.1 mm.; and with a load of 4500 ft.-lbs., to 111 mm. In young men at sea-level Dill and collaborators found that the alveolar oxygen pressure during physical exertion does not

necessarily change and that if it does the direction cannot be predicted; that is, it may either rise or fall, but during exertion at an altitude of 10,000 feet they found the alveolar response always showed a rise in oxygen pressure. Their maximum rise was 13.8 mm. The response of our boys, even at sea-level, was always a positive one similar to the anoxic experience at high altitudes.

Oxygen debt. The oxygen debt determined after 5 minutes of work was never clearly larger than has been obtained with young men. The boys, however, as they grew in size went less into debt for oxygen. After a period of 6 months J. B.'s debt after carrying a load of 6000 ft.-lbs. for 5 minutes was reduced from 1210 to 870 cc. and that of J. H. from 1085 to 910 cc. The growth increase in strength and possibly to some extent the factor of training account for the smaller oxygen debts. The debt was always paid promptly; by J. B. in 4 minutes, by R. B. in 7 minutes, and by J. H. between the 6th and 11th minutes.

TABLE 3

Two series of observations on R. B. made one year and four months apart

LOAD OF WORK	FIRST SERIES				SECOND SERIES			
	Respira- tory minute- volume	Oxygen consump- tion per minute	Pulse rate per minute	Oxygen pulse	Respira- tory minute- volume	Oxygen consump- tion per minute	Pulse rate per minute	Oxygen pulse
<i>ft.-lbs.</i>	<i>liters</i>	<i>cc.</i>		<i>cc.</i>	<i>liters</i>	<i>cc.</i>		<i>cc.</i>
Rest	6.8	243	107	2.3	8.2	305	86	3.5
1,500	16.6	754	141	5.3	21.1	900	132	6.8
3,000	22.1	997	165	6.4	27.4	1,200	146	8.2
6,000	35.0	1,425	197	7.2	50.1	1,860	164	11.3

Growth differences. R. B. grew rapidly while under observation. In November 1932 he was 5 feet and 3 inches tall and weighed 87 pounds. Early in April 1934 he was 5 feet 9 inches tall and weighed 116 pounds. Some comparisons of his response to physical exertion at the two periods have been tabulated in table 3. His resting intake of oxygen rose in the year and 4 months as much as 62 cc., and during work a larger delivery of oxygen to the active muscles was made for each load of work. For a load of 6000 ft.-lbs. the intake of oxygen was increased 435 cc. per minute. His respiratory minute volume also became larger during rest and under exertion. The most notable difference occurred when the load of 6000 ft.-lbs. was carried; the breathing at the earlier age was only 35 liters and at latter age, 50.1 liters per minute. The reactions of the heart, as shown by the pulse rate, were more favorable during the second series of experiments. The pulse frequency was less during both rest and physical exertion. Here again the greatest difference was found for the load of 6000 ft.-lbs., with the pulse rate 197 at the earlier age and only 164 at the latter age. During

the first series of experiments R. B.'s oxygen pulse was at all times less than in the other boys, but at the time of the last series it had augmented so that it was almost up to the level found among young men. Schneider found that the oxygen pulse for young men, when carrying a load of 6000 ft.-lbs., ranged between 11.7 and 12.8 cc. R. B. in the second series of experiments with a load of 6000 ft.-lbs. had an oxygen pulse of 11.3 cc. We have shown in an earlier paper that the output of blood by the heart per beat by our boys was less than that of adults. The oxygen pulse data for R. B. suggest that his output of blood per beat was still somewhat less than that of adults, but that in a year and 4 months his heart had definitely increased its stroke volume.

Steady state. In these experiments the work proceeded until the subject felt he could continue no longer. It is generally admitted that a steady state is reached when the oxygen demand is adequately met. A steady state implies a relatively constant total ventilation, respiratory rate, absorption of oxygen, discharge of carbon dioxide, pulse rate, and internal environment.

With the load of 4500 ft.-lbs. each of the boys attained an excellent degree of steadiness in the various respiratory and metabolic responses, even though they were unable to continue the work for the same length of time. In none of them did the respiratory and metabolic data account for the fatigue that terminated the work period. That heart fatigue may have played a part in endurance is suggested by the high rates of J. B. and R. B., but this does not seem a probable explanation for J. H. whose pulse rate steadily rose but reached only 164 beats. R. B.'s pulse rate reached 210 beats the last minute of work.

A summary of the data for two of the boys while carrying a load of 6000 ft.-lbs. is given in table 4. It is evident that here again, all the respiratory responses reached a steady state while the pulse rate failed to do so. Since the heart rate alone increases progressively throughout the period of work it may be concluded that the fatigue of these boys was in large measure associated with inability of the circulation to meet the demands of the active muscles.

A striking feature in all the experiments on the steady state was that the respiratory quotient, after the early minutes of work, came back to the pre-exercise level. This indicates that during work the body continued to oxidize the same foods that it was using during rest before work began.

Recovery from a load of 6000 ft.-lbs. Each boy was asked to carry this load as long as earlier experiments had indicated would be near his limit of endurance; hence R. B. worked for 5 minutes, J. H. for 10 minutes, and J. B. for 15 minutes. If we assume, as claimed by Solandt and Ridout, that the respiratory metabolic changes are the last to disappear during recovery then these boys made an extremely rapid recovery. This might

be interpreted to mean that the boys were in excellent training. Liebenow found that as five subjects trained their recovery rate diminished after a standard exertion. Our boys, like most American boys, were fond of outdoor play and were therefore active during after-school hours; but none of them indulged in regular strenuous activity. We shall show, however, that so far as boys are concerned, it is a mistake to assume that the respiratory metabolism is the last change to disappear in the recovery process. We find when metabolism has returned to the pre-exercise level that the frequency of heart beat is still above its resting level.

A typical record of recovery is given for J. B. in table 5. In this experiment J. B. contracted an oxygen debt of 870 cc. as he carried a load of 6000 ft.-lbs. for 15 minutes. In another experiment with the same load in

TABLE 4

MINUTE	RESPIRATION RATE	RESPIRATION—MINUTE-VOLUME	OXYGEN ABSORBED PER MINUTE	RESPIRATORY QUOTIENT	PULSE RATE	OXYGEN PULSE	VENTILATION EQUIVALENT
--------	------------------	---------------------------	----------------------------	----------------------	------------	--------------	------------------------

6,000 ft.-lbs. of work per minute by J. B.

		<i>liters</i>	<i>cc.</i>			<i>cc.</i>	<i>liters</i>
Rest	17	9.9	324	0.88	96	3.5	3.1
1st	30	17.8	925	0.72	150	6.2	1.9
2nd	34	32.3	1,459	0.88	172	8.5	2.2
4th	37	35.3	1,554	0.90	184	8.4	2.3
7th	40	39.7	1,645	0.90	190	8.7	2.4
10th	42	39.9	1,764	0.85	192	9.2	2.3
13th	41	38.2	1,667	0.87	198	8.4	2.3

6,000 ft.-lbs. of work per minute by R. B.

Rest	15	5.2	243	0.78	90	2.4	2.1
1st	25	22.8	1,156	0.80	154	7.5	2.0
3rd	33	39.8	1,574	1.00	208	7.6	2.5
5th	36	39.9	1,586	0.93	224	7.1	2.5

which the steady state was studied, the "lag" in the absorption of oxygen until the maximum intake was obtained indicated an oxygen debt of 1010 cc. It appears, therefore, that in the experiment recorded in table 5 J. B. reached and maintained a steady state. Judging from other experiments we estimate that his intake of oxygen during the last minute of work was approximately 1660 cc. This fell to 1021 cc. for the first minute after work and continued to fall about as rapidly during the second minute and then more slowly during the next two minutes, after which it was back to the pre-exercise level where it remained fairly constant during the next 18 minutes of observation.

The other two boys showed much the same oxygen recovery. J. H. with a pre-exercise resting intake of 308 cc. was back to 298 cc. by the

11th minute and maintained this level for the next 8 minutes of observation. R. B. made a complete recovery in 7 minutes.

The recovery in oxygen consumption by our boys corresponded in time to that observed by Marsh and Murlin in boys 14 to 16 years of age, when they carried a load of 2170 ft.-lbs. for from 5 to 20 minutes. Their recovery required 7 or 8 minutes. Marsh and Murlin, on the other hand, found that when they prolonged the period of observation of recovery as much as 15 minutes there was an apparent decreased absorption of oxygen. There is a divergence of opinion regarding the after effect. After vigorous exercise of short duration Sargent finds that recovery in men is extremely rapid, especially in the 10 minutes immediately after exercise; but that the consumption of oxygen remains above the pre-exercise resting level for some time. Hebestreit likewise found a maintained higher resting level of oxygen intake after exercise, which he attributes to a secondary stimulus of metabolism. Marsh in a study of 19 Olympic wrestlers found, after severe

TABLE 5

Recovery of J. B. after carrying a load of 6,000 ft.-lbs. for 15 minutes

TIME	RESPIRATION RATE	RESPIRATION— MINUTE- VOLUME	CO ₂ OUTPUT PER MINUTE	O ₂ INTAKE PER MINUTE	RESPIRA- TORY QUO- TIENT	VENTI- LATION EQUIVA- LENT	TIDAL AIR	PULSE RATE	OXYGEN PULSE
<i>minutes</i>		<i>liters</i>	<i>cc.</i>	<i>cc.</i>			<i>cc.</i>		
Rest	13	6.90	232	287	0.81	2.4	460	88	3.3
1	29	24.95	938	1,021	0.92	2.4	860	142	7.2
2	25	12.25	383	392	0.98	3.1	490	115	3.4
3-4	16	9.13	278	303	0.92	3.0	564	106	2.9
5-7	16.5	6.97	221	270	0.82	2.6	422	106	2.6
8-12	12.5	7.38	242	290	0.83	2.5	600	102	2.8
13-23	13	6.60	213	279	0.76	2.4	504	96	2.9

exertion, that the oxygen intake is depressed below normal for a period of 2 to 18 hours. Jahn, on the other hand, finds that the oxygen consumption in some cases rises, in others remains fairly steady, and in others falls.

The output of carbon dioxide per minute, as was to be expected, became somewhat subnormal as soon as the breathing began to subside. In J. B. this occurred after the 4th minute, in R. B. after the 6th, and in J. H. after the 12th minute. Later the depression in the carbon dioxide output tended to recover. For R. B., whose pre-exercise output of carbon dioxide was 191 cc., the low period during recovery extended from the 7th through the 13th minutes when it averaged 165 cc.; while from the 14th through the 19th minutes the average output was 181 cc. The gas analysis of expired air showed clearly this same swing in carbon dioxide output. For J. B. the percentage of expired CO₂ during the control rest period was 3.67, by the 3rd minute after exercise it was lowered to 3.32, for the next 4 minutes it averaged 3.45, and for the 8th through the 13th minutes it

rose to an average of 3.57. The other boys reacted similarly. The evidence is clear in two of our boys that depression in the output of CO_2 during recovery was of short duration and probably in no instance exceeded 20 or 30 minutes.

The respiratory quotient was, as is usual after strenuous exertion, high for several minutes. It then slowly fell to normal or even subnormal as contrasted with that of the control period that preceded exercise. J. H. had an R.Q. of 0.79 before work and of 0.78 during the 12th through the 20th minutes of recovery. R. B. whose R.Q. during the control period was 0.78 showed the low level of 0.74 from the 7th to the 20th minutes of the recovery study. J. B. (table 5) developed the R.Q. depression after the 13th minute and had an average of 0.76 for the next ten minutes, after which observations were discontinued. Marsh and Murlin invariably found that the R.Q. was lowered by exercise.

The minute-volume of breathing during the steady state for the load of 6000 ft.-lbs. averaged about 40 liters. It fell in all the boys during the first minute of recovery to between 20 and 25 liters and was back to the pre-exercise level within from 5 to 11 minutes. In 2 of the boys there was a period when it was slightly subnormal; in R. B., with a pre-exercise minute-volume of 5.63 liters, the average during the 7th to 13th minutes of recovery was 5.25 liters and the average during the 14th through the 19th minutes rose to 5.52 liters.

The ventilation equivalent for all three of the boys indicates that the respiratory metabolic balance was quite completely restored within the time we studied the recovery. Early in the period of recovery the minute-volume of breathing is distinctly out of proportion to the amount of oxygen that is consumed. The disproportion is slowly redressed, but eventually the pre-exercise proportion is fully restored. Thus the V.E. of J. B. (table 5) preceding exercise was 2.4, it rose to 3.1 the second minute of recovery and then slowly during the next 11 minutes returned to the pre-exercise level and there remained during the following 10 minutes of further observation. The V.E. for the three boys was fully restored to normal within from 7 to 13 minutes.

The recovery in the frequency of breathing was the most prompt of all the respiratory factors observed. In the case of J. H. the recovery was complete by the 3rd minute, in R. B. during the 4th minute, and in J. B. during the 8th minute. No depression in the frequency below that of the control period was observed.

The recovery of the 6 respiratory factors studied by us was completed first by the frequency of breathing, next in order came the usage of oxygen, then the ventilation equivalent, this was followed by the respiratory minute-volume, and lastly by the carbon dioxide equilibrium. The respiratory quotient in two of the boys was still depressed when observa-

tions were stopped. Hebestreit in a study of adults found that the ventilation first attains the resting state, followed by oxygen consumption and lastly by the carbon dioxide elimination.

A study of the frequency of the heart beat and the oxygen pulse brings out certain interesting facts regarding the recovery from the effects of strenuous physical activity. During the last minute of the work period under the load of 6000 ft.-lbs. the pulse rate was 192 for J. B., 196 for J. H., and 204 for R. B. In none of the boys was the pre-exercise rate restored in the period of observation. The pre-exercise rates for J. B., J. H., and R. B. were 88, 96 and 95; and at the end of the period of observation of 20 to 23 minutes the rates were 96, 114 and 106, respectively.

The oxygen pulse during and immediately after exertion is much larger than it is during rest. In our boys, after carrying the 6000 ft.-lb. load, the oxygen pulse was subnormal by the end of the second minute in each. The reaction of J. B., as shown in table 5, was similar to that of R. B., each reaching the lowest value between the 5th and 8th minutes. After this each made some recovery, but the oxygen pulse was still subnormal at the close of the period of observation. In J. H. the oxygen pulse reached the lowest value during the 12th minute and remained at that point during the period of observation. There are possibly two interpretations of the changes in the oxygen pulse during the period of recovery; these may be indicative either of variations in stroke volume of the heart or of variations in the unloading of oxygen from the blood as it flows through the tissue capillaries. The former is probably the interpretation that applies to our data. From this fact, and from the delayed return of the pulse rate, it may be concluded that the effects of exertion were more profound on the circulation than on respiration and metabolism and that recovery occurs more slowly in the circulatory than in the respiratory factors.

SUMMARY

A linear relationship between the consumption of oxygen and load of work was not in evidence. With light loads the intake of oxygen was too large and with heavy loads too small.

The resting rate of consumption of oxygen increased with the growth of two boys and at the same time the delivery of oxygen during strenuous work was augmented.

During rest the minute-volume, frequency, and depth of breathing of pre-adolescent boys correspond to that of adults.

The amount of air breathed per minute and the depth of breathing during exertion are smaller among boys than among adults, while the frequency of breathing is greater among boys.

The respiratory dead space during physical exertion increases less in boys than in adults.

The ventilation equivalent for oxygen is somewhat larger for boys than for men.

During physical exertion the pulmonary alveolar oxygen pressure invariably rose, the rise ranging upwards to 12 mm. Hg. Among men the alveolar oxygen pressure ordinarily shows no regular change.

The oxygen debt was never large. After six months of growth the debt with a heavy load was reduced.

Growth resulted in more favorable oxygen intake, in a larger lung ventilation, in a slower pulse rate, and in a larger oxygen pulse, both during rest and exertion.

In work carried to fatigue a steady state was ordinarily reached in all respiratory and metabolic factors; but the pulse rate, while maintaining a fairly steady state for a while, always showed some further accelerations with the onset of fatigue.

In recovery the metabolism returned to the pre-exercise level before the pulse rate. The slow return of the pulse rate and oxygen pulse indicates that strenuous exertion more profoundly disturbs the circulation than it does respiration and metabolism.

REFERENCES

- DILL, D. B., H. T. EDWARDS, A. FÖLLING, S. A. OBERG, A. M. PAPPENHEIMER, JR. AND J. H. TALBOTT. *J. Physiol.* **71**: 47, 1931.
- DILL, D. B., J. S. LAWRENCE, L. M. HURXTHAL AND A. V. BOCK. *J. Biol. Chem.* **74**: 313, 1927.
- DOUGLAS, C. G., J. S. HALDANE, Y. HENDERSON AND E. C. SCHNEIDER. *Phil. Trans. Roy. Soc., London, Ser. B* **203**: 185, 1913.
- HEBESTREIT, H. *Pflüger's Arch.* **222**: 738, 1929.
- HENDERSON, Y. AND H. W. HAGGARD. *This Journal* **73**: 193, 1925.
- JAHN, D. *Klin. Wchnschr.* **9**: 1757, 1930.
- KNIPPING, H. W. AND A. MONCRIEFF. *Quart. J. Med.* **1**: 17, 1932.
- LIEBENOW, R. *Ztschr. Ges. Exper. Med.* **59**: 49, 1928.
- MARK, R. E. *Arbeitsphysiol.* **2**: 129, 1929.
- MARSH, M. E. AND J. R. MURLIN. *Skand. Arch. Physiol.* **49**: 182, 1926.
- SARGENT, R. M. *Proc. Roy. Soc. B.* **100**: 440, 1926.
- SCHNEIDER, E. C. *This Journal* **97**: 353, 1931.
- SCHNEIDER, E. C. AND C. B. CRAMPTON. *This Journal* **114**: 473, 1936.
- SOLANDT, O. M. AND J. H. RIDOUT. *Proc. Roy. Soc. B* **113**: 327, 1933.

AN EXPERIMENTAL ANALYSIS OF COAGULANT ACTIVATION

JOHN H. FERGUSON

From the Department of Physiology and Pharmacology, University of Alabama School of Medicine

Received for publication March 16, 1936

In view of *a*, the demonstrable existence (questioned by some authorities, 23) of small quantities of antithrombin (15) and heparin (17) in normal blood plasma, *b*, their marked anticoagulant effects in vitro and in vivo, Howell (12-18) has insisted on a coagulation theory in which the phospholipids are denied any other rôle than that of neutralizing the antithrombic agents. The contrary viewpoint, extant since Morawitz (22), has especially been supported by Bordet (1, 2) and by Mills (19-21). The last named author made special efforts to establish the fact that calcium ions alone were insufficient to activate prothrombin, cephalin being equally necessary. Since the experimental evidence was difficult to obtain and, as far as we are aware, has not been conclusively confirmed, we have been impelled to re-study the problems involved, with an especially careful approach to control of technique. The enquiry has been extended to include an experimental analysis of the following coagulants: 1, plasma prothrombin; 2, platelets; 3, corneal-, and 4, crystalline lens extracts. The eye tissues were chosen because they are *physiologically* bloodless and hence avoid the criticism applicable to other tissue coagulants, viz., the possibility that part, at least, of their clotting powers may be due to the gross blood therein contained (24).

Experimental technique. Anesthetised dogs were bled into $\frac{1}{10}$ volume of isotonic trisodium citrate solution (3.8 per cent, 11) via a paraffined cannula in the femoral artery. The clear plasma obtained after repeated centrifugation was *rapidly* aspirated through a sterile Berkefeld "V" or "N" filter. Goddard's (10) denial of Cramer and Pringle's (4) contention that such "deplateletization" abolished plasma coagulability was abundantly confirmed.

Prothrombin-free fibrinogen was prepared from the above plasma by $Mg(OH)_2$ adsorption followed by at least two "saltings" with ammonium sulphate and a final precipitation with sodium chloride. The purified fibrinogen dissolved readily in distilled water and gave very rapid and firm clots on the addition of active thrombin. The ability of the fibrinogen solution to withstand cephalin plus calcium for 24 hours at 38°C. without clotting was a rigorous proof (Mills') of the complete absence of prothrombin.

Prothrombin was prepared from defibrinated (54°C.) Berkefeld plasma by Howell's acetone method (17, 3). These prothrombin solutions showed an unexpected stabil-

ity in that they could be kept for several days in the ice-chest with but little loss of potency. Fresh solutions, however, were used in all the cited experiments.

Platelets, carefully separated from corpuscles by differential centrifugation, were washed repeatedly with isotonic sodium citrate and freshly suspended in distilled water or 0.9 per cent NaCl solution.

The clear *cornea*, and also the *crystalline lens*, were dissected from the dogs' eyes freshly post-mortem. After rinsing in saline the tissues were dried rapidly in an air current at room temperature and stored in clean dry bottles. Extracts were made some hours before use by macerating in sterile salt solution.

Boiled extracts were obtained by boiling the various solutions for one minute. *Benzene extractions* were carried out by simple shaking in a separatory funnel with 3 to 5 volumes of cold benzene (6, 20).

Cephalin was prepared from calf brain by an extended process of purification aimed to secure freedom from *a*, proteins; *b*, fats; *c*, cholesterol; *d*, glycolipids, and *e*, lecithins. The acetone-insoluble material was finally purified by repeated (cold) absolute alcohol precipitation from ethereal solution. The pure white deposit from the final precipitation was preserved under a large volume of absolute alcohol (which protected it from oxidation). Just before use a small quantity of the alcoholic suspension (sediment) was evaporated to dryness in a tared flask over a boiling water bath. The residue was resuspended in distilled water. A 1:2,000,000 (final) dilution of a 3-months old cephalin speeded up the clotting of fibrinogen by Ca-prothrombin from $8\frac{1}{2}$ minutes to 1 minute.

Coagulation times were determined in 8 mm. coagulation test tubes immersed in a water bath thermostatically kept at body temperature (38°C.). Since interest centered in the activation of coagulant rather than the completion of clotting (the tubes were usually invertible in a relatively short time later), the recorded times denote commencement of coagulation. In order to facilitate comparisons, measured quantities were employed throughout. The dilution variable (*v. infra*) was minimised by permitting "activation" of coagulants for several minutes at 38°C. before adding the fibrinogen. All tubes were observed over a period of 2-3 days, although very few clots were formed after the first day and the "0" (no clot) sign in the tables refers to the 24-hour reading unless otherwise specified. With the rare exceptions noted, all clots were complete within a relatively short time from commencement. The cited results were substantiated by many hundreds of tests representing a searching investigation of the process of coagulant activation.

RESULTS. A. *The cephalin factor in the activation of prothrombin.* Cephalin + calcium, at all stages in the preparation of prothrombin-free fibrinogen, gave quicker clots than calcium alone. Traces of prothrombin, too small to produce coagulation in the presence of calcium salts only, still caused good clots when cephalin was also added. The final fibrinogen preparation failed to coagulate in 24 to 48 hours with calcium + cephalin, but it was clotted *a*, with variable tardiness (10 min.-2 hrs.) by Ca-prothrombin; *b*, speedily (5-10 min.) by calcium + cephalin + prothrombin, added separately (dilution effect), and *c*, with great rapidity (5-15 sec.) when these three reagents were permitted to interact for 5 to 15 minutes prior to the addition of the prothrombin-free fibrinogen.

Benzene-extracted prothrombin-free fibrinogen gave no clot with calcium and cephalin, alone or in combination. Ca-prothrombin caused the

appearance of an incomplete clot overnight, as compared with a 5 to 10 second clot with Ca-cephalin-prothrombin. Benzene extraction of the prothrombin, also, removed the slight capacity for activation by calcium alone. Cephalin — Ca — b.e. prothrombin gave a 30-second coagulant. Tested against ordinary (unextracted) fibrinogen, this b.e. prothrombin gave $3\frac{1}{2}$ minutes' clotting when calcium salts were added, and 100 seconds' clotting when activated by cephalin + calcium.

Thrombin, prepared by the interaction of prothrombin, calcium, and cephalin, could not be inactivated by simple benzene extraction (even overnight), but still gave 30 to 40 second clots (on the following day) when tested against benzene-extracted and ordinary fibrinogens.

TABLE 1
Clotting reactions of coagulants. Dog 11. 38°C.

COAGULANT	FIBRINOGEN, IN THE PRESENCE OF					
	(a) Nil	(b) CaCl ₂ (Cephalin 24 hrs. later)		(c) Cephalin (Ca, 16 hrs. later)		(d) Ca + cephalin
1. Prothrombin.	0	1 $\frac{3}{4}$ hrs.		0	Few minutes (<8 min.)	45 seconds
2. Platelets*.....	0	Overnight (>3 hrs.)		0	1 $\frac{1}{2}$ hrs.	Overnight (>3 hrs.)
3. Cornea*.....	0	20 $\frac{1}{2}$ hrs.		0	3 $\frac{1}{2}$ hrs.	2 hrs.
4. Lens*.....	0	0†	1 hr.	0	Overnight (>9 hrs.)	Overnight (>3 hrs.)
5. 0.9 per cent NaCl (control).....	0	0	0	0	0	0
	A.	B.	C.	D.	E.	F.

* Weak extracts.

† Confirmed.

The loss of the ability of *benzene-extracted prothrombin* to form coagulant with calcium alone was restored not only by the addition of cephalin (v. supra) but also my means of *a*, the benzene extractives (evaporated to dryness and resuspended in distilled water), and *b*, extracts of washed platelets and eye tissues (v. infra).

Poor (e.g., aging) prothrombin preparations, inactive on simple recalcification, were often found to recover their activity in the presence of the cited reagents. The effect of cephalin was studied carefully and it was noted that the re-activation was at first slow but speeded up remarkably after 2 to 3 hours (38°C.) just before reaching the maximal degree of activation (a 3–5 min. thrombin).

Benzene extraction experiments on *citrated plasma*, before and after Berkefeld filtration, showed a progressive loss of coagulative function (on simple recalcification). These recalcified plasmas were rapidly coagulated

by cephalin or platelets and gave good but slower clots with the benzene extractives.

We have observed that the *various lipoid solvents* can act on plasma in one of two ways. Most of them (e.g., alcohol, ether, acetone) definitely

TABLE 2

Clotting reactions of benzene-extracted (B.E.) coagulants. Dog 11. 38°C.

COAGULANT * (BENZENE EXTRACTED)	BENZENE-EXTRACTED FIBRINOGEN, IN THE PRESENCE OF			
	(a) Nil	(b) CaCl ₂ (cephalin 12 hrs. later)	(c) Ca + cephalin	
1. *Plasma prothrombin.....	0	0	Few minutes (<5 min.)	30 seconds
2. *Platelet extract.....	0	0 (12 hrs.)	$6\frac{3}{4}$ hrs.	Overnight (>2 hrs.)
3. *Cornea extract	0	0 (12 hrs.)	Overnight (>11 hrs.)	24-36 hrs.
4. *Lens extract.....	0	0 (12 hrs.)	Overnight (>11 hrs.)	24-36 hrs.
5. 0.9 per cent NaCl.....	0	0	0	0
	A.	B.	C.	D.

TABLE 3

Clotting reactions of boiled coagulants. Dog 11. 38°C.

REAGENT	FIBRINOGEN + CaCl ₂		FIBRINOGEN + CaCl ₂ + PROTHROMBIN	B.E. FIBRINOGEN + CaCl ₂ + B.E. PROTHROMBIN
	(a) Alone	(b) 6 hrs. later, + cephalin		
1. Boiled prothrombin....	0	Few minutes* (<5 min.)	5 minutes	Flocculent clot after 6 hours
2. Boiled platelets.....	0	Overnight (>6 hrs.)	10 seconds	15 seconds
3. Boiled cornea.....	0	2 hours	10 seconds	30 seconds
4. Boiled lens.....	0	Overnight (>6 hrs.)	25 minutes	20 minutes
5. 0.9 per cent NaCl (control).....	0		$1\frac{3}{4}$ hours	0
6. Cephalin (control).....	0		45 seconds	30 seconds
	A.	B.	C.	D.

* Another prothrombin (boiled 1 min.) + Ca + cephalin + fibrinogen (added simultaneously)—C.T. = 55 min.

precipitated and denatured the proteins as evidenced by the gross turbidity and irreversible loss of coagulative functions. A smaller group, exemplified by benzene, yielded plasmas of but slightly increased opalescence. These plasmas kept well and recovered their coagulative functions on

adding cephalin or materials containing "available" sources of that phospholipid.

B. *An experimental analysis of coagulant activation.* Parallel experiments (table 1) were performed on 1, plasma prothrombin; 2, washed platelets; 3, corneal-, and 4, crystalline lens extracts. None of these had any coagulative (thrombic) function prior to activation. Calcium salts alone were ordinarily sufficient to convert them into coagulant, *except* in the case of the lens extract, which needed cephalin in addition. Cephalin + calcium always gave better coagulants, i.e., maximal activation. In the absence of added calcium, cephalin had no activating power even in the case of the eye extracts which might be presumed to contain some calcium salts.

Benzene extraction of the 4 coagulant precursors always removed the capacity for activation by calcium alone, but cephalin, added subsequently, restored the status quo, and even activated the b.e. lens extract. Calcium + cephalin, incubated with the precursor material for several minutes prior to the addition of fibrinogen, gave optimal results. Since ordinary fibrinogen contains relatively large amounts of "available" phospholipid (v. infra), it was imperative to perform the foregoing tests on benzene-extracted fibrinogen (table 2).

After *boiling* the reagents, calcium alone was insufficient for thrombin formation. Cephalin, in addition to calcium, however, gave potent coagulants, even in the case of the lens extract. Indeed, boiling caused a considerable increase in the activity of the thrombins obtained (table 3).

Cephalin-like action. On testing the ability of the various materials to accelerate the clotting of prothrombin-free fibrinogen by a Ca-prothrombin preparation which required $1\frac{3}{4}$ hours to cause clotting, it was found that platelets and corneal extract reduced the clotting time to less than a minute, while lens extract had also a definite effect (59 min.). With *boiled* reagents an even more striking acceleration of clotting was observed (10 sec. with platelets and cornea, 25 min. with lens extract). Almost identical values resulted on adding the respective boiled materials to a system containing benzene-extracted prothrombin and fibrinogen (which remained unclotted on simple recalcification). The resemblance to the action of cephalin is striking (table 3). The adjuvant effect of boiled prothrombin is also noted in the cited table. It is suggested that Howell's prothrombin is poor in, but not quite devoid of, "available" phospholipid. In common with the other coagulant precursors it yields a considerable increase in this factor on boiling (heat denaturation).

DISCUSSION. Plasma prothrombin. The careful avoidance of contact with injured tissues during collection of the blood and the completeness of removal of the formed elements by centrifugation and ultrafiltration strongly support the view that prothrombin originates from the plasma

(globulin-) proteins. It is inconceivable, in our opinion, that it could have a cellular (e.g., platelet) origin under the conditions of the present experimentation.

Although, as *ordinarily prepared*, prothrombin yields an active coagulant in the presence of calcium alone, the further addition of cephalin greatly enhances and stabilizes the thrombic activity. In ageing preparations or when a mere trace of prothrombin is present, added cephalin, given sufficient time, may be essential for the demonstration of the ability to form coagulant. Since we may fairly assert that the absence of antithrombic agents was secured by the processes of purification, it follows that the clot-facilitating action of cephalin must represent a *direct* effect on the activation of the coagulant. The benzene extraction experiments demonstrate the successful removal of a clotting factor, detectable in the extractions, but best replaced by cephalin and tissue extracts (particularly boiled extracts). Benzene extraction of the test fibrinogen is an essential part of the technique. The data afford strong support for the conclusion that a phospholipid factor (identified with free or dissociable cephalin), which can be removed by sufficiently vigorous shaking with cold benzene, is as essential as the calcium ion in the direct activation of plasma prothrombin. The ability of thrombin, once formed, to resist the inactivating influence of benzene suggests that the cephalin enters into a firmer (? chemical) union with the prothrombin (and calcium ?) during the process of thrombin formation.

Coagulant activation. The experimental analysis of the process of activation of the 4 coagulant precursors, viz., plasma prothrombin, platelets, corneal-, and lens extract, emphasizes the underlying similarity of the process in each case. Thus, 1, all require activation; 2, added calcium salts are essential, and calcium alone suffices to activate all *untreated* preparations except lens extract; 3, cephalin, in addition to Ca greatly improves the coagulant potency in all cases, and causes clotting power to appear in *a*, lens extract, and *b*, benzene-extracted reagents. The data support the conclusions that cephalin is as necessary as calcium for the direct activation of *all* these coagulants, and, when calcium appears to act alone, it is because the materials (including the fibrinogen) contain what we may conveniently term "available" phospholipid. The evidence favors the thesis that the coagulant (thrombin), in all cases consists of protein (? pseudoglobulin), phospholipid (? cephalin) and calcium components. *Until it has been shown possible to prepare a phospholipid-free thrombin capable of producing the typical coagulation of phospholipid-free fibrinogen, with the aid of calcium alone, we must believe that thrombin is essentially a cephalin-protein (complex or) compound.*

Quantitative aspects of coagulant activation. The minor differences between the reactions of the products studied are interpreted as expressions

of variability in the proportions of the three components named. On adding the missing factor(s), a coagulant is formed in each case. By keeping one factor fixed and adding an excess of the other two, the maximal clotting effect obtainable from the fixed factor can be estimated in terms of the clotting time, since it has been shown repeatedly (5, 8) that C.T. is inversely proportional to concentration to coagulant (thrombin) formed.

In the absence of analytical data on *a*, protein; *b*, phospholipid, or *c*, calcium (without which a large variable due to *dilution* is uncontrolled), the preliminary computations of table 4 are expressed in terms of hypothetical units per cubic centimeter of the particular material with which we have been experimenting. Five-tenths cubic centimeter (measured) quantities

TABLE 4
Relative strengths of factors in various coagulants

COAGULANT	PROTEIN FACTOR (PRO- THROMBIN- LIKE)	LIPID FACTOR (CEPHALIN- LIKE)	LIPID FACTOR (IN BOILED REAGENT)	CALCIUM FACTOR
1. Prothrombin (ex plasma).....	44.5	0.05?	0.09?	0
2. Platelet suspension.....	0.37	0.05	133.3	0
3. Corneal extract.....	2.5	4.2	66.7	? negligible trace
4. Lens extract.....	0.1	0	100.0	? negligible trace
5. Fibrinogen solution.....	0	9.5		0
	A.	B.	C.	D.

Calculation:

$$2 \times \frac{1000}{\text{C.T. (seconds)}} = \text{"units" per cubic centimeter}$$

A. *Prothrombin factor*: Obtained on prothrombin-free fibrinogen (5) in presence of excess of cephalin + calcium.

B, C. *Lipid factor*: Obtained on benzene-extracted fibrinogen (prothrombin-free) in presence of benzene-extracted prothrombin + calcium.

D. *Calcium factor*: Obtained on fibrinogen (prothrombin-free) in presence of added prothrombin + excess of cephalin.

of coagulant precursor solution were used for each cubic centimeter of test fibrinogen. Results were calculated by means of the formula:

$$2 \times \frac{1000}{\text{C.T. (seconds)}} = \text{"units" per cubic centimeter}$$

The method is based upon that by which Fischer (8, 9) investigated thrombic activity, and, by analogy, we shall speak of *a*, prothrombin units; *b*, cephalin units, and *c*, calcium units. The clotting times (not cited) represent the best values in a large number of tests.

The preliminary quantitative data suggest, tentatively (in the absence of control of the dilution variable), that the protein factor of plasma pro-

thrombin is large enough in comparison with that of platelets, cornea, and lens material to explain its occurrence in the last three materials as the possible result of diffusion from the blood stream, via the lymph and tissue fluids in the case of the eye tissues.

Since the hypothesis of the three essential components of thrombin is supported by experiments which avoid the needless complication of oxalation whereby Mills (21) sought to differentiate between coagulants of plasma and tissue origin, the assumption of such differences is unnecessary. The complex effects of oxalate, of course, require independent elucidation (7).

It is probable that the clotting of blood normally owes its inception to irreversible changes (denaturation phenomena) in platelets, in tissue elements, and, perhaps to a greater extent than is usually admitted, in the plasma "protein complex" (23). These changes render "available" enough cephalin (phospholipid) to overcome the normal protective mechanism afforded by the antithrombic factors (heparin and antithrombin), and to enter into the formation of the thrombin complex (calcium also being necessary). We believe that the rôle of the antithrombic factors is to take care of situations, physiological and pathological, in which a sudden increase in the "available" cephalin in the blood stream threatens, since calcium and prothrombin are ever-present, to produce thrombin and the attendant risk of intravascular clotting. Under "physiological" situations may be instanced the *lipemia* following meals, the correlation of which with a phase of increased coagulability of the blood is well recognized. Of pathological conditions, *hemophilia* is the most interesting. An elucidation is suggested on the basis of an exaggerated stability of lipoproteins (sources of cephalin) retarding the liberation of the necessary cephalin. Our viewpoint may be termed a "cephalin availability theory." It admits of a definite solution of the coagulation problem along physico-chemical lines.

SUMMARY

Experiments are offered in support of an hypothesis which regards coagulants, whether of plasma or tissue origin, as protein-cephalin-(calcium) compounds. A quantitative technique is outlined for evaluating the relative strengths of these essential components of the coagulant complex.

It is tentatively suggested that the protein factor, in all cases, originates from the plasma globulin (prothrombin).

The necessity for cephalin in the direct activation of the precursor material directs attention to "available" sources of that phospholipid. The great increase in cephalin "units" after boiling is believed to indicate that the denaturation phenomena following the shedding of blood (and lysis of platelets and injured tissues) make "available" more cephalin than can be

taken care of by the antithrombic factors. Howell's theory of the rôle of the antithrombic agents is modified in favor of a *cephalin availability theory* on the basis of which an explanation of the normal in vivo incoagulability of the blood and of the clotting time changes *a*, after meals, and *b*, in hemophilia is vouchsafed.

We wish to thank Professors R. McBurney and E. B. Carmichael for freely placing at our disposal the respective facilities of the Bacteriology and Biochemistry Departments. To Prof. W. H. Howell (Johns Hopkins) and Dr. C. A. Mills (Cincinnati), for their continued interest and helpful suggestions, we render our sincerest appreciation.

REFERENCES

- (1) BORDET, J. Ann. Inst. Pasteur, **34**: 561, 1920.
- (2) BORDET, J. (Herter lect.) Johns Hopkins Hosp. Bull. **32**: 213, 1921.
- (3) CEKADA, E. B. This Journal **77**: 512, 1926.
- (4) CRAMEŔ, W. AND H. PRINGLE. Quart. J. Exper. Physiol. **6**: 1, 1913.
- (5) EAGLE, H. J. Gen. Physiol. **18**: 531, 1935.
- (6) FERGUSON, J. H. Proc. Soc. Exper. Biol. and Med. **31**: 929, 1934.
- (7) FERGUSON, J. H. Proc. Soc. Exper. Biol. and Med. **34**: 797, 1936.
- (8) FISCHER, A. Biochem. Ztschr. **259**: 67; **264**: 178, 184, 1933; **270**: 250, 275, 1934.
- (9) FISCHER, A. Japan. J. Exper. Med. **13**: 223, 1935.
- (10) GODDARD, C. H. This Journal **35**: 333, 1914.
- (11) HITCHCOCK, D. I. AND R. B. DOUGAN. J. Gen. Physiol. **18**: 485, 1935.
- (12) HOWELL, W. H. The Harvey Lectures, Ser. XII, 272, 1916-17.
- (13) HOWELL, W. H. (Pasteur lect.) Proc. Inst. Med. Chicago **5**: 139, 1925.
- (14) HOWELL, W. H. Physiol. Reviews **15**: 435, 1935.
- (15) HOWELL, W. H. This Journal **29**: 187, 1911.
- (16) HOWELL, W. H. This Journal **31**: 1, 1912.
- (17) HOWELL, W. H. This Journal **35**: 474, 1914.
- (18) HOWELL, W. H. This Journal **77**: 680, 1926.
- (19) MILLS, C. A. Am. J. Med. Sci. **172**: 501, 1926.
- (20) MILLS, C. A. Chinese J. Physiol. **1**: 235, 435, 1927.
- (21) MILLS, C. A. This Journal **95**: 1, 1930.
- (22) MORAWITZ, P. (Review) Ergebn. d. Physiol. **4**: 307, 1905.
- (23) PICKERING, J. W. The blood plasma in health and disease. 1928.
- (24) SMITH, H. P., E. D. WARNER AND K. M. BRINKHOUSE. This Journal **107**: 63, 1934.

THE ACTION OF A SINGLE VAGAL VOLLEY ON THE HEART OF THE EEL AND THE TURTLE

ERNST FISCHER

From the Laboratory of the Marine Biological Association, Plymouth, England; the Marine Biological Laboratory, Woods Hole; and the Department of Physiology and Pharmacology, Medical College of Virginia, Richmond, Va.

Received for publication May 13, 1936

For several years I used as a class demonstration of inhibitory nerve effect, a vagus-heart preparation of the eel because of its readiness to respond to single induction shocks. Although from such records only marked inhibition could be detected, it was obvious that the inhibiting effect depends on that phase of the normal heart cycle at which the stimulus was applied. As an explanation I used the generally accepted assumption that in consequence of the arriving vagal impulses A.C. (an acetylcholine-like substance) is freed and later disappears by diffusion or destruction by an esterase. The dependence of the inhibitory effect on the beat cycle, in the case of a single vagal volley, I believed due to a high rate of A.C. formation and its rapid disappearance. It was not until the summer 1934 that I found the opportunity to study in detail this time relation of assumed A.C. formation and disappearance. The results obtained were so peculiar that I repeated the experiments the next summer, only to confirm my previous findings, which I later also found in turtles.

METHOD. Most of the experiments on eels (*Anguilla* and *Conger*) were performed with the brain and the upper part of the spinal cord pithed through a small opening in the skull, the wound having been filled with absorbent cotton to minimize blood loss. A constant flow of sea water was kept running through the gills. Such a preparation remained in good circulatory condition for several hours. In a few experiments an excised vagi-heart preparation was perfused by eel Ringer (Fischer, 1926). Both preparations reacted in the same way to vagal stimulation. In most of the experiments both vagi were stimulated simultaneously with induction shocks, the heart beats were recorded by the suspension method either from the ventricle or the auricle on superficially smoked drums rotating at a speed of 3 to 5 cm. per second.

In turtles (mostly *Pseudemys*), after pithing them in the same manner as the eels, a hole 3 cm. in diameter was cut with a trephine into the plastron exactly above the heart. The movements of both the auricles and of

the ventricle were recorded at the same time by the suspension method either on a superficially smoked drum or on a photo-kymograph simultaneously with the electrocardiogram taken by direct leads. The vagi were stimulated in the neck by condenser discharges; each vagus alone or both simultaneously.

EXPERIMENTAL RESULTS. *The dependence of the chronotropic effect on the rhythmic mechanism of the pacemaker.* The results in experiments on eels as well as on turtles, when the ventricular beat was recorded, could be easily divided into two classes: 1, lengthening of the first influenced ventricular cycle up to 75 per cent, and 2, lengthening of the first cycle over 100 per cent or even over 200 and 300 per cent. In experiments in which the auricular beat was used as indicator, and the contraction of the interjugular part of the sinus in eels or of the vena cava superior in turtles was well expressed in the auricular curve, it became obvious that the extremely long ventricular heart cycle was often due not only to a chronotropic effect upon the pacemaker, but mainly to a blocking of the conduction from the pacemaker to the auricles (fig. 1). Therefore, the true chronotropic effect upon the pacemaker can be detected only in those experiments in which the time relation of the beats of the pacemaker can be seen in the auricular tracing or in the electrocardiogram in experiments on turtles.

Measuring carefully such records from the eel experiments, typical results were obtained as shown in figure 2. The vertical lines represent the occurrence of excitation in the pacemaker, the beginning of the jugularis movement being taken as the moment of excitation. The arrows mark the approximate moment at which the vagal impulse reaches the pacemaker. These moments have been calculated from the moments of stimulation by experimentally determined conduction times of the vagal nerves employed and by the shortest latent period obtained by direct stimulation of the pacemaker. Such a calculation, however, can only be an approximate one with an error of at least 0.05 of a cycle length; but this error is always the same throughout the whole series of stimulations in one experiment. The crosses indicate the time at which a normal beat of the vena jugularis would occur, if the cycles were not prolonged. The height of the crosses above the basal lines indicates the amount of inhibition in percentage of normal cycle length. The figures on the left of the basal lines are the times, in fractions of a normal cycle,

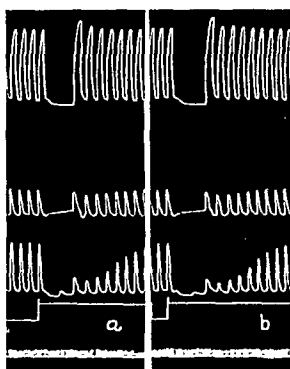


Fig. 1. Turtle. Upper curve: ventricle, then left auricle, right auricle, signal, time in $\frac{1}{3}$ second. Right vagus, condenser discharge. Stimulation in b about $1/10$ cycle later than in a. 3/18/36.

between the arrival of the volley and the moment at which the next normal beat would appear.

A vagal volley arriving at about the moment of a normal beat does not influence at all the cycle between this beat and the next, but has a moderate but marked effect upon the later cycles. If the volley arrives later than $\frac{1}{3}$ cycle length after the initiation of a normal beat, but earlier than $\frac{1}{4}$ cycle before the next expected beat, the cycle in progress is lengthened to a great extent and the later cycles are only very little influenced. If the volley reaches the heart later in the cycle, less than $\frac{1}{4}$ cycle before the

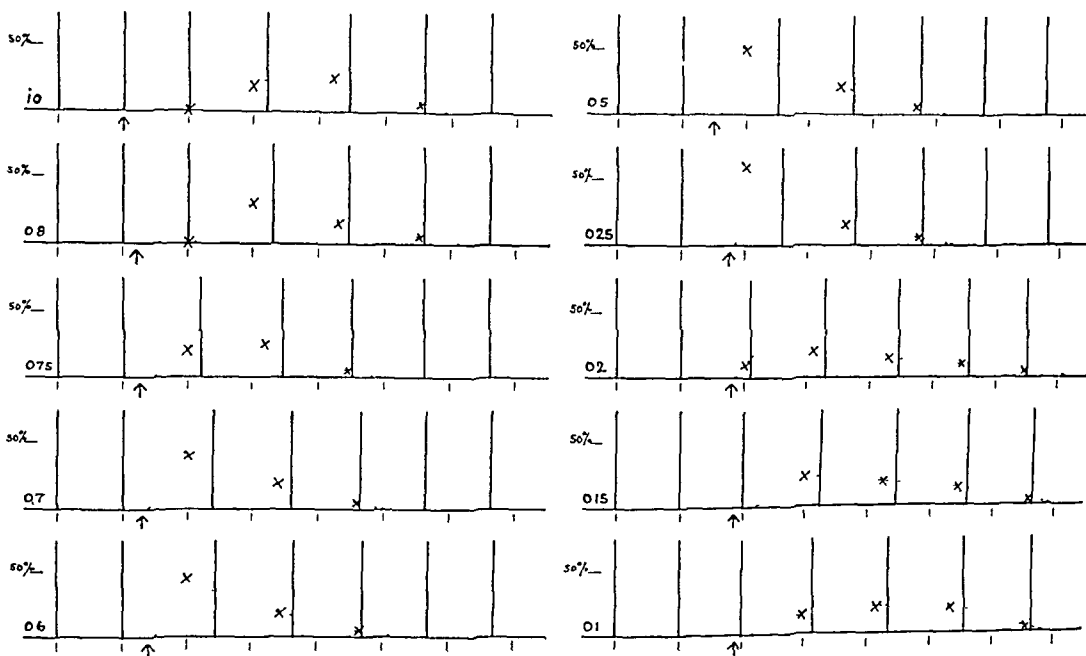


Fig 2 Eel, both vagi stimulated simultaneously by single, just maximal condenser discharges. Vertical lines: excitation of the pacemaker. Arrows: the moments, at which the vagal impulses reach the pacemaker. Crosses: strength of inhibitory effect. Time in normal cycle lengths. For further explanation see text. 4/29/34.

next beat, the initiation of that beat is less delayed, and also the lengthening of the next cycle is less than one might expect. The later cycles are influenced to a relatively great extent.

It is impossible to draw through the experimentally found points identical curves representing the A.C. formation and disappearance for the different stimulations. Assuming that the retardation of a beat is proportional to the A.C. present in or around the pacemaker at the moment a normal beat would start, one should expect identical A.C. curves when the volleys reaching the pacemaker at various phases of the cardiac cycle would always produce, after a constant latent period, a definite amount

of A.C. at a constant rate. The hypothetical dotted curves in figure 3 are drawn in such a way as to allow a relatively simple explanation of the experimental facts.

A volley arriving about half way between two beats liberates after a short latent period a relatively large amount of A.C. at a high rate. In these cases the declining part of the A.C. curve may represent the diffusion and destruction of the A.C. When the volley reaches the pacemaker at the moment of a normal beat or shortly after, A.C. is set free only after a

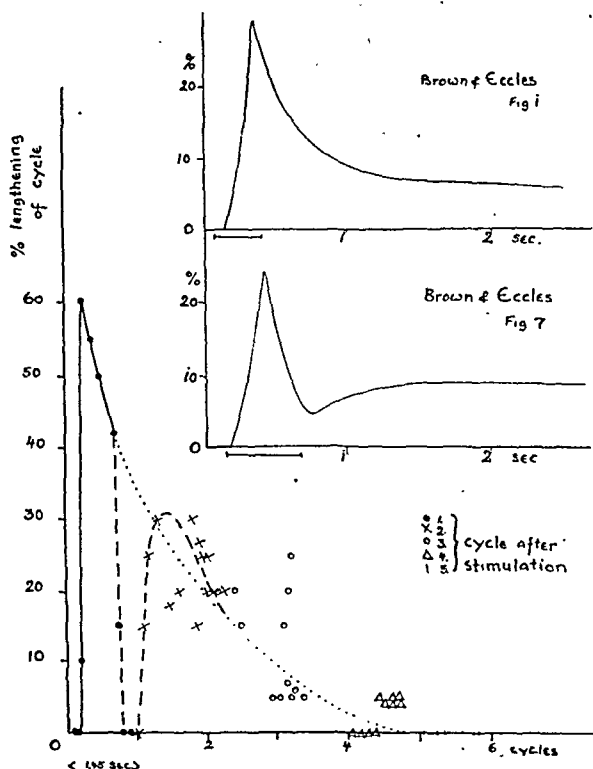


Fig. 3. Same experiment as in figure 3, all experimental points plotted in one graph, the moment of arrival of the vagal volley taken as zero time. Upper inserted curve: single wave type A.C. curve; lower inserted curve: double wave type A.C. curve, (Brown and Eccles, 1934). Further explanation in text.

very long latent period and at a much slower rate, but for a longer time, so that A.C. is still being liberated in the later part of the curve. If a volley arrives at any other time of the heart cycle, both the quick and the slow liberating mechanism come into play, each acting only in part.

Brown and Eccles (1934); who investigated the effects of single vagal volleys upon the cat heart, used another way of representing the experimental results. They plotted all data gained by stimulations at various points in the heart cycle, using the moment of stimulation as zero time

and measuring the degree to which a beat was retarded. In most of their experiments they were able to draw through their numerous points a very smooth curve, as shown in the upper inserted curve of figure 3. Representing my data in such a way (fig. 3), a similarly shaped curve can only be drawn through the very first points. The dotted part of that curve is obviously contradictory to the experimental facts. The broken line would represent the experimental findings more accurately, but its last part is indeterminable. In my experiments such a method of representation cannot give a definite A.C. curve because the formation of A.C. is not constant, but varies with the phase of the heart cycle at the moment of stimulation. Nevertheless this attempt of representation is not only useful in summarising the result of a series of stimulations, but helps—as will be discussed later (p. 604)—to demonstrate that we are not dealing with a peculiarity of the eel, but with a more general principle.

Stopping the bloodflow or the perfusion through the heart, or cutting away the ventricle, had no significant influence upon the chronotropic effect of a single vagal volley. Of all measures tried, only vagal fatigue had a distinct influence. The vagal fatigue was produced by several prolonged tetanic stimulations of both vagi and allowing a sufficiently long time to elapse for the normal heart cycle to be restored. In all experiments of this kind the dependence of the vagal effect on the heart cycle was not altered, but in most of the experiments, after fatigue, the early quick liberation process decreased in importance while the late slow one increased (fig. 4). In consequence of this shift in importance, the trough in the A.C. curve becomes more narrow, but never disappears. In a few cases, after vagal fatigue the trough did not reach the basal line completely, due to the fact that a vagal volley arriving at the pacemaker at just about the moment of a normal excitation has a small inhibiting effect upon the following beat, whereas before fatigue such a stimulation had no effect on that beat.

Experiments on turtles gave results indicating the same dependence of the vagal effect on the cycle phase. In the experiment of figure 1, which was recorded at about $\frac{1}{6}$ of the normal recording speed, it is easily seen that the second stimulation, although it occurs only approximately $\frac{1}{10}$ of a cycle phase later, has a quite different effect upon the pacemaker as expressed by the movements of the sinus venosus, which are clearly seen in the tracing of the right auricle. Measuring out curves taken at a higher speed, the same double mechanism of quick and slow A.C. liberation was found in all experiments (fig. 5). This dependence on the cycle phase was never completely absent, but was not always so great as in the eel experiments.

Is the dependence on the pacemaker activity a specific feature of the chronotropic vagal effect or present also in other vagal actions? The distribution of

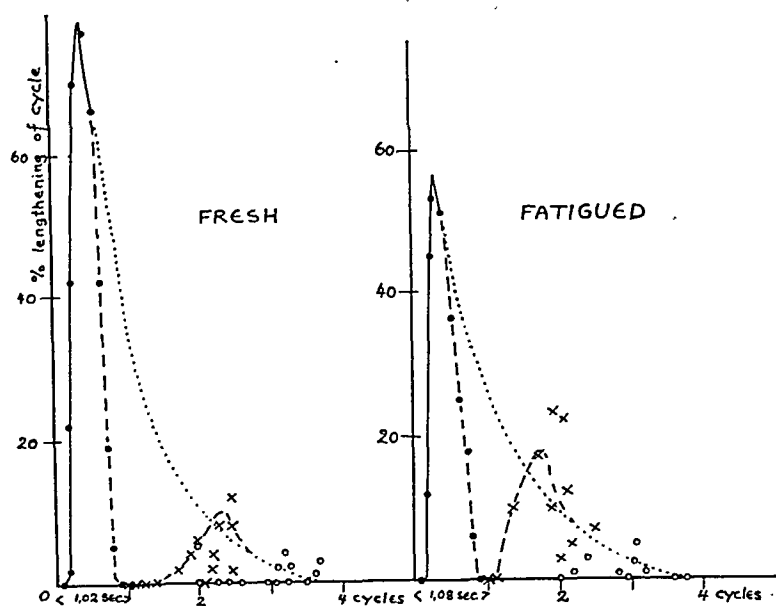


Fig. 4. Eel. A.C. curve before and after vagal fatigue. Both vagi stimulated simultaneously. 6/28/35.

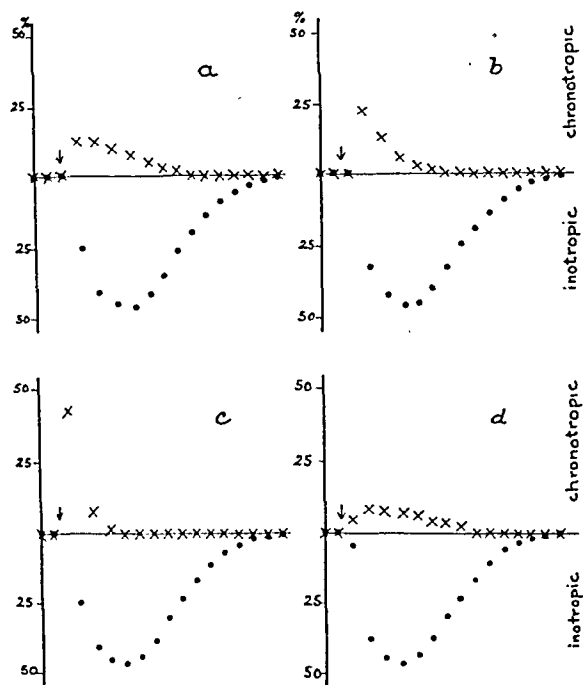


Fig. 5. Turtle. Chronotropic and inotropic (right auricle) effects. Time between arrival of vagal volley and next expected beat in a = $\frac{1}{2}$, in b = $\frac{1}{2}$, in c = $\frac{3}{4}$ and in d = $\frac{1}{4}$ of a normal cycle length. Right vagus, condenser discharges of equal strength. 12/10/35.

the action of the right and the left vagus upon the different parts of the eel and turtle heart I found, generally speaking, in accordance with previous work of others (Gaskell, 1883; Garrey, 1911, 1912; Gilson, 1932, 1933; Lee, 1935; Fredericq, 1936). In those turtle experiments, in which the movements of the different parts of the heart were recorded simultaneously, it could be demonstrated that, even when the chronotropic effect was markedly dependent on the cycle phase, the inotropic effect upon the right auricle was completely independent of the cycle phase (fig. 5). Even when the vagal volley reaches the pacemaker at such a

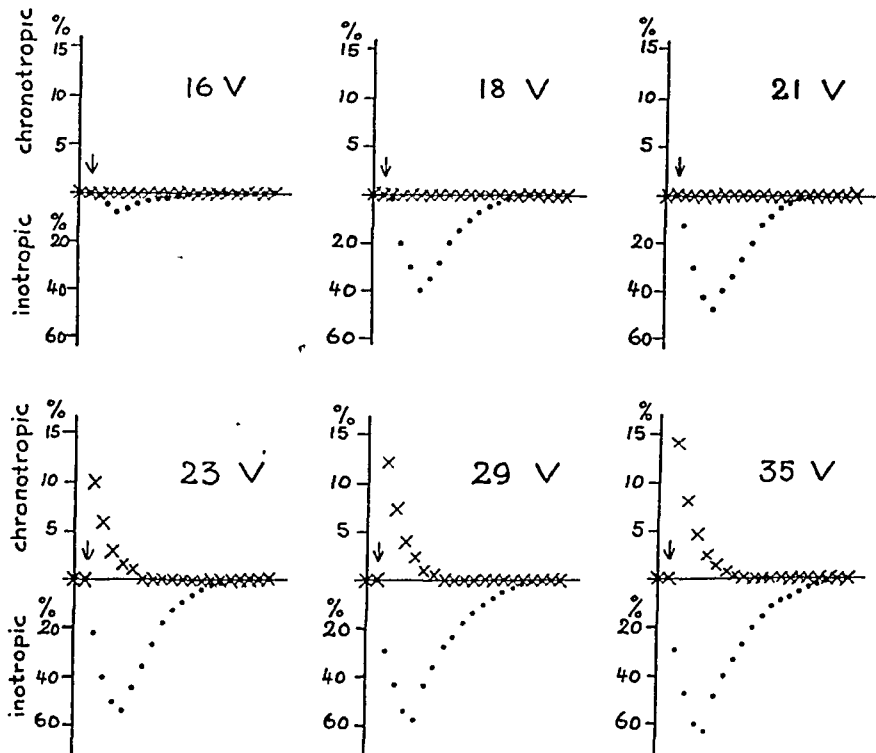


Fig. 6. Turtle. Chronotropic and inotropic (right auricle) effects. Stimulations always $1/5$ cycle length after a normal beat. Discharges of a $1\mu\text{F}$ condenser, voltage increased. Right vagus only. 12/3/35.

time that the chronotropic effect is much delayed, its maximum action is reached distinctly earlier than the maximum of the auricular inotropic effect, the time relations of which correspond completely to those reported by Gilson (1932).

In five out of 27 turtles it was possible to demonstrate clearly by a threshold method that in the right vagus different fibres convey the impulses for chronotropic and inotropic action (fig. 6). In these experiments there was not the least indication that the intensity of the chronotropic effect has any influence upon the inotropic effect in the right auricle.

In not a single one of my experiments, either with the eel or the turtle, were single vagal volleys able to block completely the conduction from the auricle to the ventricle, even using both vagi simultaneously. The lengthening of conduction time between right auricle and ventricle, as found by measuring the electrocardiograms, occurs more or less parallel to the inotropic effect upon the right auricle and was independent of the cycle phase.

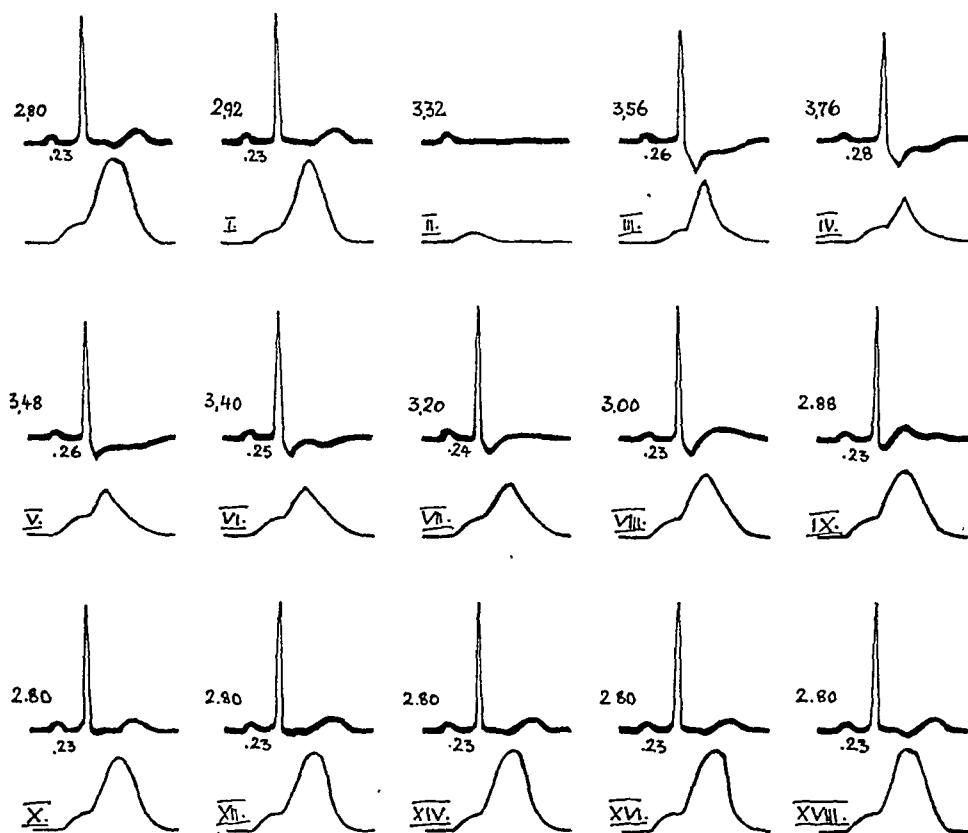


Fig. 7. Turtle, ventricle cut away completely; one electrode on right vena cava, other on tip of right auricle. Lower curve mechanogram of right auricle with cava movements recognizable. Numbers above electrogram: distance of pacemaker wave from preceding pacemaker wave in seconds. Numbers under electrogram: pacemaker-auricle conduction time. Roman numbers: number of beats after stimulation of right vagus. 12/10/35.

The action of the pacemaker was just detectable in a few of the electrograms obtained from turtles, from which the whole ventricle was removed, and in which one of the electrodes touched the right vena cava and the other the tip of the right auricle. However, when after vagal stimulation apparently no conduction to the right auricle occurred, the electric representation of the pacemaker was not altered (fig. 7). The graphic records

of the movements of the sinus venosus did not disappear completely. Besides this a complete blocking of the conduction occurred most irregularly, often being present after a stimulation of certain strength at a certain cycle phase, then missing again. But when this blocking was present, it was obvious that this dromotropic (pacemaker to auricle) effect occurred later than the maximal chronotropic effect, even when the latter was maximally delayed by the choice of the cycle phase at which the stimulus was applied (fig. 7).

The action of eserine, atropine, and acetylcholine. Eserine injections (0.1 to 0.2 mgm.) into the vena jugularis externa of the turtles increased and prolonged to a marked extent the inotropic effect of vagal stimulation upon the auricle. Its chronotropic effect is only slightly altered, but its dromotropic effect upon the conduction from the pacemaker to the auricles is increased.

Small doses of atropine sulfate (0.01–0.1 mgm.) had generally the same depressing or abolishing effect upon the chronotropic and inotropic vagal actions. When only the inotropic action was depressed, the dependence of the chronotropic action on the cycle phase was still present. But as in the experiment with vagal fatigue, the quick A.C. liberating process was more depressed than the slow one. Smaller doses of atropine had no effect at all, and I could never observe an increased excitability of the vagal mechanism as described by Gremmels (1935) for cats and rabbits after very small doses of atropine.

Injection of acetylcholine (0.0003–0.001 mgm.) exerted a marked inotropic action upon the auricle, but had only little influence upon the pacemaker and upon the size of the movements of the right vena cava although the conduction to the auricles might be blocked completely.

DISCUSSION. *The double mechanism of A.C. liberation for the chronotropic vagal action*, as found in my eel and turtle experiments, seems to represent a general principle. Brown and Eccles (1934), whose paper I saw only after my eel experiments had been finished, report that they did not always obtain smooth A.C. curves in their cat experiments. In a relatively large number of cats they found A.C. curves of a double wave type, as represented in the lower inserted curve of figure 4. Brown and Eccles measured the time in seconds, instead of in normal cycles as I did, and since they used as zero time the moment of stimulation instead of the arrival of the volley, they were probably not aware of the fact that the deepest point of the trough, which produces the double wave curve, is always just about one cycle length later than the arrival of the volley. Fortunately they have stated for each series the cycle length, which I have indicated in their curves copied in figure 4. Furthermore, it is obvious from their data that the single wave type curve always occurs in animals if the normal cycle is short, but the double wave type when the

normal cycle is long. This explains, too, why they were able in some cases to change the double wave curve into a single wave curve by previously started and maintained weak stimulation of the accelerator nerves.

In experiments of Brown and Eccles the double wave curve represents the same phenomenon as in my experiments, as is shown by the further fact that in the case of A.C. curves of the single wave type, all their experimental points show almost no deviation from the drawn curve, while in the double wave type A.C. curves, only the first part of the drawn curve is readily determined by the experimental points.

Brown and Eccles were not able to offer a good explanation for the trough in their curves. They exclude with good reasons 1, a transient change of the pacemaker to another center; 2, a transient acceleration introduced within a single inhibitory effect; 3, after-discharge from postganglionic neurones; 4, a secondary A.C. accumulation due to diffusion from surrounding tissues. All their reasons mentioned to exclude these explanations are valid also for the experiments reported in this paper. I can only add that I could not find any evidence in the eel that any heart accelerating nerve fibres or mechanisms exist at all, which was to be expected according to the report of McWilliam (1883). In some of the turtle experiments a distinct accelerating effect was occasionally present, when using supermaximal stimulation. In those rare cases, however, the accelerating action preceded clearly the vagal action, as the first one or two cycles were shortened and the strength of the auricular beats increased, while the next cycles showed distinct lengthening and the beats were diminished in size.

The final explanation of Brown and Eccles for the trough in their A.C. curves is rather unsatisfactory. They refer to experiments by Eccles and Hoff (1934) with very early subsequent beats, which according to them are a comparable phenomenon. Therefore, they conclude that the pacemaker, like other rhythmic centers, contains "inhibitory components," and that these might be dissociated from the remainder of the center, consequently freeing it from the inhibitory action.

This explanation assumed that the trough in the A.C. curve is present for each single stimulation. It is evident, especially from the eel experiments, that the A.C. curve for a single stimulation (fig. 3) never exhibits any sign of a trough, while in a graph summarizing the results of a whole series of stimulations a trough reaching the base-line results (fig. 4). This is due to the fact, as pointed out already, that the curves for a single stimulation are not identical, so that when plotted together a trough must originate just about a cycle length later than the moment of the arrival of the vagal volley. All beats which could be delayed at that time are beats which have been influenced by a vagal volley preceding them by approximately one cycle length. The individual A.C. curves for these stimulations start only after a very long latent period and with only a gentle

slope. In consequence, the beats occurring at the time of the trough are not altered at all, or only very little.

The question remains: why do the latent period, velocity and duration of A.C. liberation depend on the cycle phase? One may suggest that the mechanism of A.C. liberation and the rhythmic mechanism of the pacemaker are linked closely together, using partly the same anatomical substrate. Thus, when a vagal volley arrives just at the moment when the rhythmic mechanism is about to free an excitation, the A.C. liberating mechanism is depressed, responding with a longer latent period and a slower velocity. However, the fact that about the same amount of A.C. is set free nevertheless is easily explained by the generally accepted assumption that preformed A.C. is released and that A.C. is not produced in consequence of a vagal stimulation.

The nature of the A.C. Recently several papers have been published (Feldberg and Vartiainen, 1935; Vogt, 1936; Brown and Feldberg, 1936; Feldberg and Guimarães, 1936) presenting evidence that probably acetylcholine is not the only parasympathetic transmitter, but that the liberation of potassium ions plays an important part in the transmission of the excitation from the preganglionic to the postganglionic fibres. These authors assume that it is the potassium ions which are freed first, and which then produce the acetylcholine liberation. Howell (1906), Howell and Duke (1908), emphasized thirty years ago that in the heart K is freed in consequence of vagal stimulation and that these K ions produce the inhibition. Lehnartz (1936), repeating Howell's experiments, came to the conclusion that in the heart the output of K is associated with the inhibitory process but is not the true transmitter of the vagus impulses. He still regards acetylcholine as the transmitter. Armstrong (1936) found that acetylcholine has a chronotropic effect on the heart of *Fundulus* embryos only after the growing nerves had reached the heart. Therefore, he assumed A.C. to be liberated only on the intramural synapses.

All of this evidence suggests a discussion of the quick and slow A.C. liberating mechanism from the point of view that the freed substances in both processes are not identical. One may assume that the quick liberating mechanism is producing acetylcholine, while the slow process liberates K ions. The fact that atropine depresses the quick mechanism more markedly than the slow one would support this assumption. Vagal fatigue depresses the quick and increases the slow process. Furthermore, eserine increases and prolongs the inotropic vagal effect much more markedly than the chronotropic effect. That the substance freed during chronotropic vagal actions is not pure acetylcholine is demonstrated by the weak action of injected acetylcholine upon the pacemaker in my own experiments, in those of Cope and Coombs (1935) in cats, and in the frog experiments of Bacq (1935). The A.C. liberated during marked vagal

inhibition of the pacemaker must reach the right auricle. Nevertheless, no influence on the size of the chronotropic effect upon the auricular inotropic effect is present (figs. 5 and 6). According to the experiments of Fredericq (1935) with quite a different method, the A.C. liberated at the sinus has only a weak influence upon other parts of the heart. Although all this evidence points out clearly that we are probably dealing with different substances in the vagal mechanism, the experimental results are still too contradictory to allow the postulation of any theory about the particular rôle these different substances play.

SUMMARY

1. In the eel the chronotropic effect of a single vagal volley is markedly dependent on the cycle phase at which the volley reaches the pacemaker. In turtles the same dependence of the vagal effect on the cycle phase is detectable.

2. It is assumed that around the pacemaker there are two mechanisms of liberation of an acetylcholine-like substance (A.C.): a quick mechanism with a short latent period, and a slow mechanism with a long latent period. The degree to which each of these liberating processes comes into play depends on the cycle phase. Vagal fatigue decreases the importance of the quick, and increases the importance of the slow A.C. liberating mechanism.

3. Neither the dromotropic nor the inotropic vagal action is dependent on the cycle phase.

4. Eserine prolongs markedly only the inotropic and dromotropic vagal effect. Atropine depresses the chronotropic effect as well as the inotropic effect. Small doses of atropine, however, depress the quick chronotropic A.C. liberation more than the slow one. Acetylcholine acts more strongly on the inotropic and dromotropic mechanism than on the chronotropic.

5. The experimental results are interpreted as indicating that the different vagal fibres affecting the heart are not only functionally distinct, but that there exist several chemical transmitters for the vagal action.

REFERENCES

- ARMSTRONG, P. B. *J. Physiol.* 84: 20, 1935.
BACQ, Z. M. *Ergebn. d. Physiol.* 37: 82, 1935.
BROWN, G. L. AND J. C. ECCLES. *J. Physiol.* 82: 211, 242, 1934.
BROWN, G. L. AND W. FELDBERG. *J. Physiol.* 86: 10 P., 290, 1936.
COPE, O. M. AND H. C. COOMBS. *Proc. Soc. Exper. Biol. and Med.* 33: 480, 1935.
ECCLES, J. C. AND H. E. HOFF. *Proc. Roy. Soc. London B.* 115: 307, 327, 352, 1934.
FELDBERG, W. AND J. A. GUIMARÃES. *J. Physiol.* 86: 306, 1936.
FELDBERG, W. AND A. VARTIAINEN. *J. Physiol.* 83: 103, 1935.
FISCHER, E. *Proc. Roy. Soc. London B.* 99: 326, 1926.
FREDERICQ, H. *Arch. Intern. Physiol.* 42: 323, 1936.

- FREDERICQ, P. C. r. Soc. Biol. Paris **118**: 1628, 1935.
GARREY, W. E. This Journal **28**: 330, 1911.
This Journal **30**: 450, 1912.
GASKELL, W. H. J. Physiol. **4**: 43, 1883.
GILSON, A. S. This Journal **100**: 459, 1932.
Proc. Soc. Exper. Biol. and Med. **30**: 625, 1933.
GREMMELS, H. Arch. Exper. Path. und Pharmacol. **179**: 360, 1935.
HEINBECKER, P. This Journal **98**: 220, 1931.
HEINBECKER, P. AND G. H. BISHOP. This Journal **114**: 212, 1935.
HOWELL, W. H. This Journal **15**: 280, 1906.
HOWELL, W. H. AND W. W. DUKE. This Journal **21**: 51, 1908.
LEE, H. M. This Journal **112**: 207, 1935.
LEHNARTZ, E. J. Physiol. **86**: 37 P., 1936.
MCWILLIAM. J. Physiol. **4**: 1 P., 1883.
VOGT, M. J. Physiol. **86**: 258, 1936.

THE EXTINCTION OF STARTLE RESPONSES AND SPINAL REFLEXES IN THE WHITE RAT¹

C. LADD PROSSER AND WALTER S. HUNTER

From Clark University, Worcester, Mass., and Brown University, Providence, R. I.

Received for publication June 11, 1936

A conditioned response ultimately disappears if the conditioned stimulus is repeated without reinforcement. This decline of the response during repeated stimulation was called experimental extinction by Pavlov (1927). The disappearance of unconditioned responses upon repeated stimulation has been observed in the laboratory by Rademaker (1931) and is common in daily life. The nearest approach to this phenomenon in spinal reflexes is "fatigue" or failure of a reflex response during tetanic stimulation (Sherrington, 1906, p. 218; Forbes, 1912). The present investigation is a study of the general phenomenon of extinction of reflex responses in terms of known physiological mechanisms.

We have conducted experiments on a startle leg response to auditory stimulation and on several spinal reflexes in response to mechanical and electrical stimulation. Electrical, observational and kymographic methods of recording were utilized, the latter with spinal animals only. White rats (4-6 months old), highly inbred descendants of Wistar stock, were used.

The startle response. In studying the startle response, a rat was placed in a zipper bag similar to that of Shadle and Skarupinski (1935), but with holes cut so that the rat's hind legs and tail protruded and, if required, a portion of the rat's back could be exposed. The animal was then loosely tied on an adjustable stand below which its legs appeared. In this position it usually remained quiet for several hours. Concentric needle electrodes were inserted into various leg muscles, usually the gastrocnemius. The animal and stand were placed inside a relatively soundproof box, and approximately one hour was allowed for adaptation before beginning the experiment. The box also contained a telegraph-key sounder and a small 1.5 volt lamp which was usually kept lighted throughout an experiment. The animal could be observed through a small glass window. Wires led outside the box to a battery and switch which actuated the sounder and to an amplifier. Muscle potentials were recorded with a Matthews oscillo-

¹ A portion of the cost of this investigation was met by a grant to W. S. Hunter from the American Academy of Arts and Sciences.

graph and loud speaker. Stimulation by the telegraph sounder consisted of a brief click which, if above threshold value, elicited a leg response. (The threshold for this response was usually higher than that for the pinna reflex.) No quantitative measurements of the sound were made, but variations in intensity were produced by altering the tension on the sounder. When repetitive stimulation was used the sounder was activated through contacts controlled by a telechron clock, otherwise stimulations were controlled by a hand switch.

An electrical response in the gastrocnemius muscle is shown in figure 1. The startle response consisted of a brief burst of discharges in the group of muscle fibers from which the potentials were recorded. Frequently each unit fired only once, but sometimes there was an after-discharge in which each unit fired rhythmically. The latency varied over a wide range for the different units in the response, but the latency of the fastest unit was usually between 15 and 25 ms.

The effect of repeating the click stimulus once every 15 seconds is shown in figures 1 and 2B. For a number of stimulations (30 in the experiment of fig. 2B) there was a response as judged by observation through the window of the box and by the disturbance in the loud-speaker. Then the response became reduced, irregular for 35 more (see 2B), and finally disappeared. Photographic records made from time to time during this extinction of the response are shown in figure 1. During the course of the extinction the response diminished in size as shown by a diminution in the duration of the after-discharge and a decrease in the number of active motor units until ultimately no units responded. As units dropped out during the extinction, their latencies did not alter appreciably and their magnitudes did not change. The effect is as if the threshold for different units became higher, each dropping out at some level without undergoing any change in its time relations.

As shown in figure 2B, during the period when the response is irregular there appear to be waves or cycles of excitability. These are shown by intervals when the response is present followed by corresponding intervals when no responses occur. The number of responses between periods of no response is reduced; i.e., the periods when no response occurs are not lengthened. Stimulation at a faster rate, once every two or five seconds, induced extinction in a much shorter time than when stimulation intervals were ten or fifteen seconds.

Once the effect is complete to the extent that approximately five consecutive stimulations elicit no responses, the extinction may persist through many minutes even though no stimuli are given. Further stimuli strengthen the extinction and the more stimuli given after extinction, the longer does the effect last. Spontaneous recovery does usually occur, however, after 15 to 20 minutes of non-stimulation.

If, after extinction is complete, general sensory stimulation occurs, such as opening the door of the box or flashing a bright light in front of the rat, the response in most cases returns immediately to the next auditory stimulus and requires numerous repetitions before it is again extinguished.

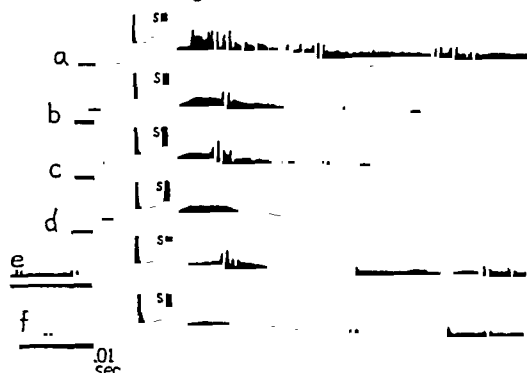


Fig. 1. Extinction of startle response in intact rat. *S*—stimulus signal. *a*, *b*, first two responses; *c*, 11th response; *d*, extinction—15th stimulus. *e*, Next successive response. Disinhibition by light occurred between *d* and *e*. Same units present as before extinction. *f*, 9th stimulus after disinhibition (extinction).

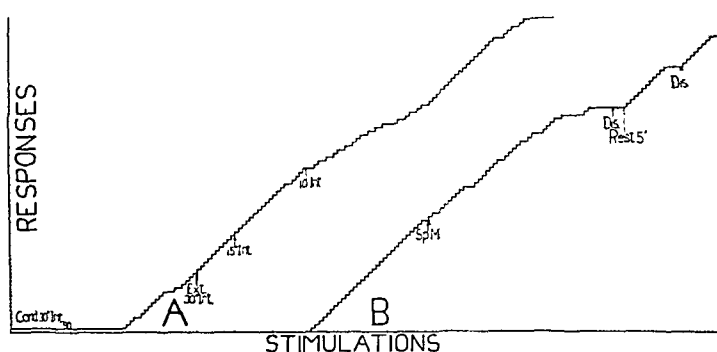


Fig. 2. "Conditioning" and extinction of startle response in intact rat. Ordinates—responses; abscissae—stimulations. Responses by observation of animal and by listening to loud-speaker disturbance.

A. "Conditioning" with sound followed by shock to foot, paired stimuli every 30 seconds. Ninety stimuli omitted from figure at 90. *Ext.* 30'' *Int.*—extinction of the conditioned response began with stimuli unreinforced, 30 second intervals. 15'' *Int.*—unreinforced stimuli at 15 seconds intervals. 10'' *Int.*—unreinforced stimuli at 10 seconds intervals.

B. Extinction of unconditioned startle response. *Sp. M.*—spontaneous movement. *Dis.*—attempted disinhibition by light. Rest 5 minutes, followed by spontaneous recovery. Second disinhibition effective because extinction not so complete as at time of first attempt at disinhibition.

This effect is shown in figures 1e and 2B. It is similar to the phenomenon called "disinhibition" by Pavlov. If the extinction proceeds to a considerable degree, a stronger stimulus is required for disinhibition than after the first few failures to respond. When the response returns, either sponta-

neously or as a result of disinhibition, the general characteristics as measured by the muscle potentials are the same as before extinction (fig. 1e). The latencies for individual units appear to be unaltered throughout the process of extinction and recovery.

In some experiments, instead of starting with a sound of an intensity which elicited a startle leg response, an intensity sub-threshold for this response was used. At each presentation the click was followed after 0.5 second by a reinforcing tetanic shock applied to the hind foot of the leg with the electrodes. In these experiments, after 75 to 100 paired presentations, a response to the sound usually appeared. The course of the establishment of the "conditioned" response is indicated in figure 2A. The nature

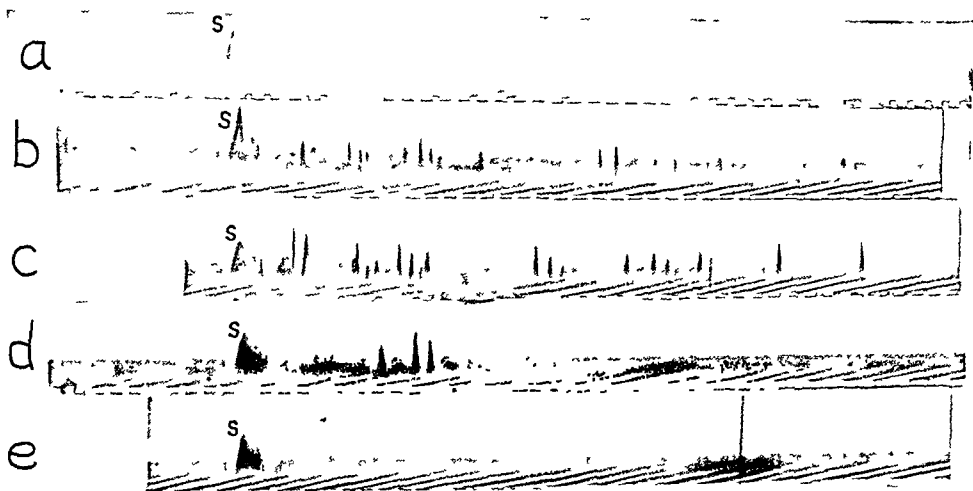


Fig. 3. "Conditioning" and extinction of startle response in intact rat. *S*—stimulus signal. Time intervals 0.01 second. a, Near beginning of conditioning, after 75 reinforced stimulation; b, during conditioning, after 20 more reinforced stimulations; c, well "conditioned" response, after 154 more reinforced stimulations; d, partially extinguished response (50 unreinforced stimuli); e, extinction complete.

of the response is shown in figure 3. The response is essentially the same as the previously described unconditioned startle response in latency, number of active units, duration of after-discharge, and frequency of individual units. Once the response was established it could be extinguished by omission of the reinforcement. The course of this extinction is similar to the extinction of the unconditioned response. In some experiments it was impossible to condition the response in approximately 150 trials. This is in keeping with the findings of many investigators who observe extreme variability among animals in their susceptibility to conditioning.

In another set of experiments the conditioning method was applied to the unconditioned startle response, using as the "conditioned" stimulus an

intensity of auditory stimulus just sufficient to evoke the response. The sound was followed 0.5 second later by a reinforcing shock. In most animals the response became irregular for a number of presentations, then it usually became stronger. The strengthening of the response by this method consisted in an increase in the number of active units and a greater duration of the after-discharge. There was no significant change in the latency of individual units.

It is questionable, in view of the nature of the response, whether any of the above experiments deal with true conditioning. It appears rather that the general excitation by the electric shock raises the excitability of the centers involved so that a sub-threshold stimulus becomes and then remains effective. The constancy of the several motor unit latencies which we have found contrasts *a*, with the decreasing latency of the gross conditioned response as found by Anrep (1920) and Hilgard and Marquis (1935); and *b*, with the increasing latency described by Switzer (1934).

The latencies of the fastest units of the startle response (15–25 ms.) are such that the response, although made by an intact animal, probably does not involve the cerebral cortex which itself shows a minimum latency of 8 ms. to auditory stimulation (Davis, 1934). Lorente de Nó (1933) has described a tensor tympani reflex to sound mediated via the inferior colliculus and superior olivary body with a total latency of 14 to 16 ms. It seems likely that the startle response involves the following path: cochlea, eighth nerve, cochlear nucleus, inferior colliculus (latency to here 2.5–3.5 ms. as determined by Kemp and Coppée (1936)) reticular nucleus in mid-brain, reticulo-spinal tract, anterior horn cells, and motor nerves to leg. Assuming 3 to 4 ms. delay in the anterior horn cells, 2 to 3 ms. for conduction on the efferent side, and 3 to 4 for end-plate delay, we are left with 4.5 to 7 ms. from the colliculus to the anterior horn cells, a path which probably involves two synapses. Previous investigators (Culler and Mettler, 1934) report the conditioning of decorticate animals, and auditory startle responses have been observed in cats in which the brain was transected through the anterior colliculi (Forbes and Sherrington, 1914).

Spinal reflexes. The preceding experiments demonstrate, we believe, extinction, reinforcement and disinhibition of an essentially collicular response. It is important, therefore, to ascertain whether these are properties of all forms of behavior (and of all nerve centers), and we have thus extended the work to include experiments on several spinal reflexes.

Chronic spinal preparations were made either by inserting a needle between the vertebrae and so transecting the cord, or, more often, by exposing the cord by the removal of two mid-thoracic vertebral dorsal arches and then transecting with a knife. Most of the animals were kept for one or two weeks, and there was a complete failure of cephalad and caudad conduction across the transection throughout the period. The following

reflexes were studied: 1, leg response to tap on the rump and to tap on the tail; 2, flexion of base of tail to tap near tip of tail; 3, tail flexion and leg response to tetanic stimulation of the tail; and 4, crossed leg response to direct electrical stimulation of the contralateral saphenous nerve. Of these reflexes the least satisfactory was the response to the tap on the rump. The tap stimulus was administered with the head of a tack held in a lever

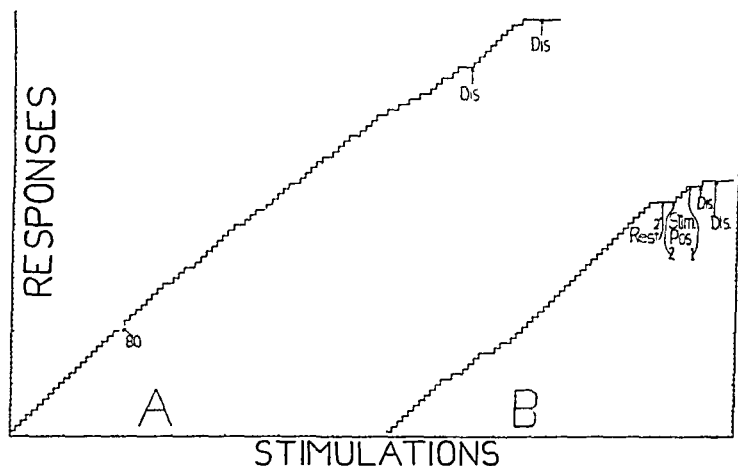


Fig. 4. Extinction of tail response in spinal rat. A, extinction to electric shock on tail. Eighty stimuli and responses omitted as shown. Dis—disinhibition by pinch; second ineffective because extinction more complete than at first. B, extinction to tap on tail. Rest two minutes—no recovery. Stim. Pos. 2—tap shifted approximately 2 mm. followed by response. Stim. Pos. 1—tap returned to first position, response still extinguished. Dis.—disinhibition by pinch.

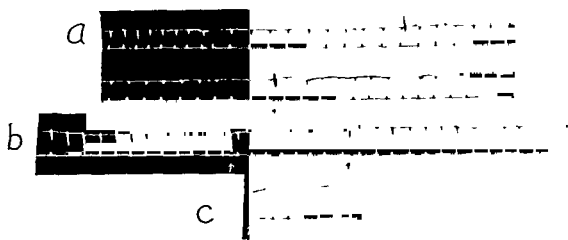


Fig. 5. Extinction of crossed leg response to electrical stimulation of saphenous nerve, spinal rat. A: stimulus near threshold, gradual diminution and ultimate extinction of response, pinch on foot at arrow caused disinhibition. B: Continuous with A. Two disinhibitions at arrows, subsequent extinction. C: Effect of same stimulus after crushing nerve centrally.

which was activated by the armature of an electromagnet. In all cases the tail was held immobile by adhesive tape at the area stimulated. Electric recording was by concentric needle electrodes placed either in the ventral base of the tail or in the gastrocnemius of one hind leg.

The records of figures 4 and 5 are typical and show that all of these responses eventually become extinguished during repetitive stimulation.

The kymograph records clearly show that as stimulation continues the response diminishes in magnitude. When the stimulus was near threshold, the spinal responses diminished and finally disappeared after fewer stimulations than when the stimulus was stronger. In fact with very strong taps and electrical stimulations, the response was frequently not extinguished, although it was reduced in size. When a response was extinguished to a given stimulus intensity, an increase in the intensity would again arouse the response.

The electrical measurements show that as the spinal responses become extinguished the number of active units and the duration of the after-discharge are reduced with no diminution or increase in the latency for a given unit. As with the startle response, the latency of the fastest unit means little because during extinction this unit may be extinguished before one which is a little slower, and after extinction of the first unit this slow one becomes the leading unit of the response.

In studying extinction with direct nerve stimulation, a spinal rat was etherized and the saphenous nerve of one leg was exposed and freed from the surrounding tissue. Insulated silver wire stimulating electrodes were placed under the nerve. The incision was closed and the rat was allowed to recover, then placed on its back in the zipper bag, tied on the platform support with its legs raised. Tetanizing shocks, 0.5 second duration, of near threshold value for the contralateral leg response, were delivered at the rate of 4 per minute by the telechron timer. The kymograph records of figure 5 are typical. Prior to the beginning of record A, the rat had received 83 shocks with no extinction. The shock was decreased, and the record shows 32 more responses followed by 3 shocks without a response. Pinching the tail, at the point marked with an arrow in the figure, disinhibited the response. Later in the experiment, the response was twice again extinguished and disinhibited (record B). In order to show that the response to the shock was neurally controlled rather than due to electrical spread or to mere mechanical jarring from the operated leg, the nerve was now pinched centrally from the electrode. Record C shows that the shock no longer produced a response in the leg.

Figures 4A and 4B also show disinhibition of spinal reflexes as a result of strong afferent stimulation resulting from pinching the tail or the foot of the rat. We have, in addition, secured records of apparent self-disinhibition. Here after the response has been extinguished and the rat has, for some reason, struggled "spontaneously," the reflex reappears to the subsequent stimuli. Such self-disinhibition may account for much of the variability found in extinction records.

INTERPRETATIVE COMMENTS. The extinction here described is considered as a central phenomenon. Evidence is unanimous that at frequencies of stimulation such as one every 10 or 15 seconds no sensory adaptation

occurs. This is certainly true for the auditory tracts (Davis et al.) and for muscle and tactile receptors. Further, the fact that we find extinction of spinal reflexes elicited by direct stimulation of the nerve shows that sensory adaptation is not involved. Similarly, there is no evidence that the motoneurons and muscles themselves show any such decline in responsiveness.

The experiments illustrated in figure 4B show that the extinction effect must occur antecedent to the final motoneurons. Here a tail response to a tap on the tail was extinguished. Then the tapper was moved 1 to 2 mm. to activate other receptors and a response of the original motor units as well as of additional motor units occurred at once to the tap in the new location. This is in harmony with the evidence recently presented by Loucks (1935) indicating that conditioning must occur antecedent to the pyramidal cells of the cortex. If this argument holds, a reflex in which an internuncial neurone occurs should not be capable of extinction. We have obtained a liminal knee jerk in a spinal cat 980 successive times in one experiment and 800 times in another with no diminution in the response (stimulation frequency 4-6 per min.). In the same animal a reflex in which internuncial neurones are involved was extinguished in 27 trials. The evidence as a whole indicates that extinction and disinhibition occur beyond the sensory neurone and ahead of the final motoneurone, presumably at some of the internuncial synapses.

What is the nature of the change in conduction that occurs during extinction? It cannot be inhibition in the Sherringtonian sense since this central inhibition lasts only 100 to 300 ms. (Creed, Denny-Brown, et al., 1932, Ch. 6). For the same reason it cannot be equilibration as described by Gerard and Forbes (1928). It is unlikely that it is fatigue in the usual sense of the accumulation of waste products and the exhaustion of reserves. The circulation is entirely adequate to prevent such effects when stimuli fall so far apart as in the present experiments. Furthermore, the effect occurs more quickly at low (near-threshold) than at high intensities of stimulation, although the stronger stimuli should produce the greater fatigue and exhaustion. In addition, the fact that strong general excitation can wipe out (disinhibit) the effect is incompatible with notions of fatigue.

Dusser de Barenne and McCulloch (1934) found an "extinction" of responses to stimulation of the motor cortex when periods of stimulation fell at intervals of approximately 13 seconds. Fatigue, or pseudo-fatigue of spinal reflexes, developed with a much higher frequency of stimulation than we have used, is a central phenomenon (Sherrington, 1906; Forbes, 1912; Lee and Everingham, 1909). Whether such "reflex fatigue" and Pavlovian "inhibition" are the same is at present a verbal matter to be settled, if at all, in terms of experimental methods employed.

The extinction process involves the gradual dropping out of active units and the diminution in the duration of their activity. The effect may persist for many minutes, and it can be eliminated (disinhibited) by general excitation. These facts indicate that extinction is a gradual lowering of the excitability; the excitability may remain low for a relatively long period, or it may be raised by disinhibiting stimuli. Conversely, *increased* excitability of long duration can be produced by reinforcement of a weak stimulus by general excitation (electric shock).

SUMMARY

A reflex startle leg response in the rat, probably mediated by the colliculus, occurs to brief auditory stimulus. When the stimulus (a click) is repeated at intervals of 10 to 15 seconds the response weakens and ultimately disappears. This extinction consists of a gradual diminution in the number of active motor units, a decrease in the duration of the after-discharge, but no change in latency for any units.

After complete extinction the response spontaneously recovers, often after 5 to 30 minutes' rest without stimulation. Recovery may occur at once if the animal becomes generally excited (disinhibition).

Reinforcement of a sub-threshold sound by a shock to the foot raises the excitability of the center so that the response appears after many paired stimulations. This "conditioned" response can be extinguished by the same method as the unreinforced response.

The following spinal reflexes can be extinguished with stimuli repeated at intervals of 10 to 15 seconds: leg and tail response to tap on tail, to tap on back, to electric shock on tail, crossed leg reflex to stimulation of saphenous nerve. After extinction the responses can be brought back (disinhibited) by strong general excitation (e.g., pinch on foot).

These effects are interpreted as being slow, semi-permanent shifts in excitability of some part of the reflex arc between the sensory neurones and the final motoneurones.

REFERENCES

- ANREP, G. V. J. *Physiol.* 53: 367, 1920.
CREED, R. S., D. DENNY-BROWN, J. C. ECCLES, E. G. T. LIDDELL AND C. S. SHERRINGTON. Reflex activity of the spinal cord. Oxford, 1932.
CULLER, E. AND F. A. METTLER. J. *Comp. Psychol.* 18: 291, 1934.
DAVIS, H. Ch. 18, *Handbook of Gen. Exper. Psychol.*, Worcester, 1934.
DUSSER DE BARENNE, J. G. AND W. S. McCULLOCH. *Proc. Soc. Exper. Biol. and Med.* 32: 524, 1934.
FORBES, A. *This Journal* 31: 102, 1912.
FORBES, A. AND C. S. SHERRINGTON. *This Journal* 35: 367, 1914.
GERARD, R. W. AND A. FORBES. *This Journal* 86: 186, 1928.
HILGARD, E. R. AND D. G. MARQUIS. J. *Comp. Psychol.* 19: 159, 1935.
KEMP, E. H. AND G. COPPÉE. *Proc. Am. Physiol. Soc.*, 91, 1936.

- LEE, F. S. AND S. EVERINGHAM. This Journal 24: 384, 1909.
- LORENTE DE NÓ, R. Trans. Am. Laryngol., Rhinol. and Otol. Soc. 1: 1933.
- LOUCKS, R. B. J. Psychol. 1: 5, 1935.
- PAVLOV, I. Conditioned reflexes. Oxford, 1927.
- RADEMAKER, G. J. Das Stehen. Berlin, 1931.
- SHADLE, A. H. AND L. SKARUPINSKI. Science 82: 335, 1935.
- SHERRINGTON, C. S. The integrative action of the nervous system. Oxford, 1906.
- SWITZER, S. A. J. Exper. Psych. 17: 603, 1934.

SPINAL VASOMOTOR REFLEXES ASSOCIATED WITH VARIATIONS IN BLOOD PRESSURE¹

C. HEYMANS, J. J. BOUCKAERT, SIDNEY FARBER² AND F. Y. HSU³

*From the J. F. Heymans Institute of Pharmacology and Therapeutics,
University of Ghent*

Received for publication June 24, 1936

It is well known that the aortic arch and the carotid sinuses constitute vascular zones sensitive to blood pressure, and are the seat of reflex regulation of cardiac frequency and vascular tone. In a publication in 1929 (1), one of us pointed to the existence of other reflex vascular zones, likewise sensitive to alterations in blood pressure, and located probably in the vascular area bounded by the arch of the aorta and the bifurcation of the iliac arteries. The present studies are concerned with the localization and rôle of these latter pressure-sensitive vascular zones.

METHOD. All experiments performed in the first part of these studies were carried out with the same basic technique. Dogs, anesthetized with chloralose, were prepared in the following manner: the spleen of a dog B was perfused by a dog A; by making anastomoses with the aid of Payr cannulae, the splenic artery of dog B was joined to the carotid artery of dog A, and the splenic vein of dog B was joined to the external jugular vein of dog A. The extrinsic innervation of the spleen of dog B remained intact. The vascular tone of the perfused spleen of dog B was registered by means of a plethysmograph. The carotid sinus nerves and the vagus-depressor (aortic) nerves of dog B were cut to exclude the reflex action of the pressure-sensitive vascular zones in the carotid sinuses and the aortic arch. The blood pressure of dog B was modified, either by hemorrhage or the intravenous injection of serum, blood or substances which cause hypertension (adrenaline, ephedrine).

In a first series of experiments, it was found, in confirmation of the observations previously made by one of us (1), that in the absence of the pressure-sensitive reflex zones in the aortic arch and in the carotid sinuses, modifications in the general blood pressure still cause inverse and com-

¹ Preliminary communication C. R. Soc. Biol. **122**: 115, 1936. The expenses of this investigation were defrayed in part by a grant from the Josiah Macy Jr. Foundation.

² Moseley Fellow, Harvard University, Boston, Mass.

³ China Foundation Fellow.



Fig. 1. Dog A, 17.0 kgm.; dog B, 19.0 kgm. Chloral-osane anesthesia. The spleen of spinal dog B is perfused by dog A and is connected with its owner (dog B) only by means of the extrinsic splenic nerves. From above downward: volume of the spleen of dog B; general blood pressure of dog A (P.A.A.); general blood pressure of dog B (P.A.B.). At 1, intravenous injection of 0.1 mgm. adrenaline into dog B. Note dilatation of the spleen of dog B in response to hypertension in spinal dog B.

Time in intervals of 3 seconds.

pensatory reactions on the vascular tone. This was demonstrated by the occurrence of dilatation of the perfused spleen of dog B in response to arterial hypertension in dog B, and constriction of the perfused spleen of dog B in response to arterial hypotension in dog B. Experiments previously carried out in this laboratory (1, 2, 3), had demonstrated that neither adrenaline nor modifications in the general blood pressure influences the tone of the vasomotor centers in a direct manner. Other possibilities were therefore investigated.

1. Another group of experiments was undertaken to determine whether the vasomotor reactions described above, in the dog deprived of the vascular zones in the aortic arch and carotid sinuses, persist in a dog deprived of his encephalo-bulbar centers.

METHOD. The technique described above was employed, with the following additions: the neck of dog B was severed at the level of the second or third cervical vertebrae by means of a special crusher (1); the trunk of dog B was kept alive by artificial respiration.

RESULTS. When variations in the arterial pressure of trunk of spinal dog B were brought about, there occurred vasodilatation of the perfused spleen of dog B when the arterial pressure in the trunk of dog B was raised by adrenaline (fig. 1), ephedrine, or pituitrin, and a vasoconstriction of the perfused spleen of dog B following a lowering of the blood pressure in the trunk of dog B.

The vasomotor tone of the spleen is therefore regulated in a neurogenic manner by alterations in the general blood pressure in an animal deprived not only of its four buffer nerves but also of its encephalo-bulbar vasomotor centers. Certain questions concerning this dependence of the vascular tone of the spleen on the general blood pressure in a spinal dog could be answered by these experiments. The possibility of a specific action of adrenaline on the spinal vasomotor centers was at once excluded, since the same effect on the spleen was obtained when other substances were used to raise the blood pressure. The possibility that the reactions in the spleen might be caused by a "washing-out" of the spinal vasomotor centers due to a sudden increase in

the spinal cord circulation in an animal with abnormally low blood pressure could be excluded by the observation that vasodilatation of the perfused spleen occurred also in experiments where the initial blood pressure

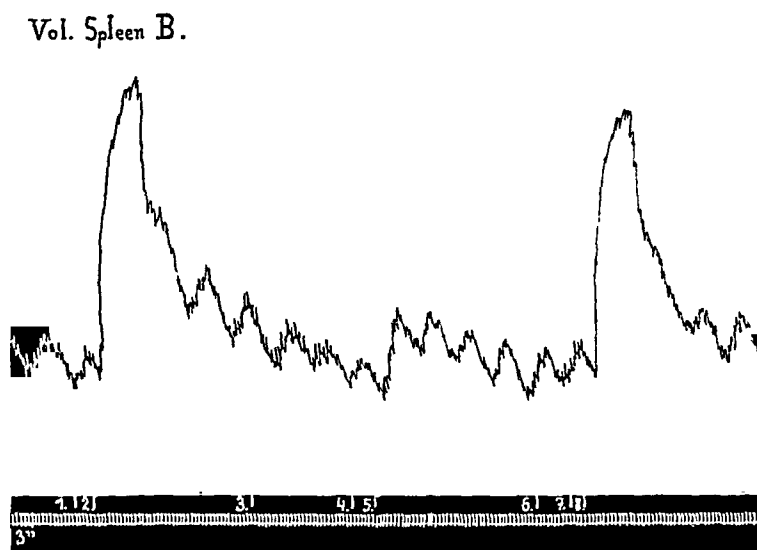


Fig. 2. Dog A, 19.0 kgm.; dog B, 22.0 kgm. Chloralosane anesthesia. The carotid sinus nerves, both vagi and the aortic (depressor) nerves of dog B have been cut. The spleen of dog B, perfused by dog A, is connected with its owner (dog B) only by means of the extrinsic splenic nerves.

The numerals refer to the following procedures carried out on dog B. At:

1. Occlusion of the aorta just *below* the origins of the celiac and superior mesenteric arteries.
2. Injection into the external jugular vein of 0.2 mgm. adrenaline. Note marked reflex vasodilatation of the spleen in response to arterial hypertension.
3. Occlusion of the aorta ended.
4. Occlusion of the aorta *above* the origins of the celiac and superior mesenteric arteries.
5. Injection into the external jugular vein of 0.2 mgm. adrenaline. Note very slight dilatation of the spleen in response to arterial hypertension.
6. Occlusion of the aorta ended.
7. Same as 1. Occlusion of the aorta just below the origins of the celiac and superior mesenteric arteries.
8. Same as 2. Injection into the external jugular vein of 0.2 mgm. adrenaline. Note marked reflex dilatation of the spleen in response to arterial hypertension. Time in intervals of 3 seconds.

was normal, and even above normal, before hypertension was artificially induced.

2. Since a direct action on the spinal vasomotor centers could be ruled out, a second hypothesis seemed permissible: could there be a reflex

mechanism comparable to that which regulates vasomotor reflexes originating in the aortic arch and the carotid sinuses?

To answer this question, in a first series of experiments, variations in the arterial pressure of a dog B were effected alternately during occlusion of the aorta above and below the origins of the celiac and the superior mesenteric arteries. Under these conditions, it was found (fig. 2) that, when the blood pressure was raised in a dog B, deprived of all other vasosensitive zones, in that portion of the aorta where the celiac and superior mesenteric arteries originate, an intense vasodilatation occurred in the perfused spleen of dog B; on the contrary, when the vascular area supplied by the celiac and the superior mesenteric arteries was excluded, and then elevation of the general blood pressure brought about, only a very slight vasodilatation of the perfused spleen occurred. It was also found that acute destruction of the spinal cord of the spinal dog caused complete disappearance of the splenic vasomotor reflexes just described.

From these experiments it appears that the vasomotor reactions in the spleen, brought about by variations in the general blood pressure, are reflex in origin, and are determined, for the most part, by the effect of blood pressure on a pressure-sensitive innervation located in the organs and structures supplied by the celiac and superior mesenteric arteries.

3. The next problem to be considered was whether these spinal vasomotor reflexes caused by variations in general blood pressure, and more especially, by arterial hypertension in the spinal dog, affect not only the vasomotor tone of the spleen, but also other vascular areas. A new series of experiments was carried out to investigate this question.

METHOD (fig. 3). The method of Delezenne was employed. The leg of a dog B, connected with the trunk of a spinal dog B only by the sciatic, crural and obturator nerves, is perfused by a dog A. By means of Payr cannulae, anastomoses are effected between the crural artery of the leg of dog B, and the carotid artery of dog A, and between the femoral vein of dog B and the external jugular vein of dog A. The vasomotor tone of the perfused leg of B is registered by the recurrent arterial pressure in the profunda branch of the femoral artery (method of Nolf), or by the lateral arterial pressure in the artery of perfusion.

RESULTS. When the general blood pressure in the trunk of B was raised, (fig. 4) vasodilatation of the perfused leg of B occurred. A return to normal blood pressure or a hypotension in trunk B caused a vasoconstriction of the perfused leg of dog B.

The arterial pressure of the spinal dog, therefore, influences by reflex means, not only the vascular tone of an abdominal viscus—the spleen, but also the peripheral vascular tone, as was noted in the perfused leg.

4. These spinal vasomotor reflexes, as well as the carotid sinus and aortic arch vasomotor reflexes were completely absent in the totally sym-

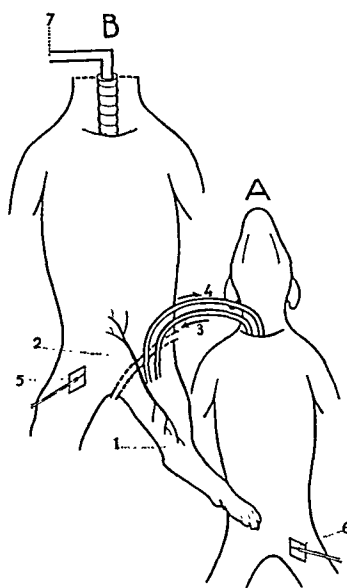


Fig. 3

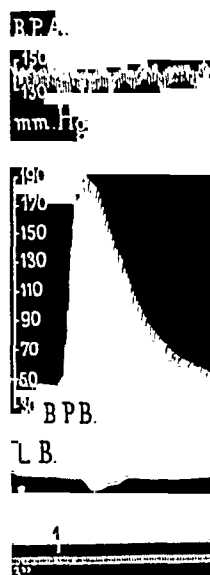


Fig. 4

Fig. 3. Diagrammatic representation of the technique employed in the perfusion by dog A of the hind leg of a spinal dog B, which is connected with its owner (dog B) only by the sciatic, crural and obturator nerves.

1. Hind leg of dog B.
2. Intact sciatic, crural and obturator nerves.
3. Arterial pathway from the carotid artery of dog A, anastomosed to the crural artery of the hind leg of dog B.
4. Venous pathway from the femoral vein of the hind leg of dog B, anastomosed to the external jugular vein of dog A.
5. Cannula in the femoral artery of the intact hind leg of dog B for the registration of the general blood pressure of dog B.
6. Cannula in the femoral artery of dog A for the registration of the general blood pressure of dog A.
7. Tracheal cannula, which is connected to an artificial respiration apparatus.

The spinal cord of dog B is severed at the level of the 2nd or 3rd cervical vertebra. The carotid sinus nerves, and both vagus and aortic (depressor) nerves have been cut.

Fig. 4. Dog A, 17.0 kgm.; dog B, 11.0 kgm. Chloralosane anesthesia. Prepared according to the technique illustrated in figure 7. The vasomotor tone of the perfused leg is measured by the lateral pressure in the hind leg of dog B. Vasodilatation—downward.

From above downward: arterial pressure of dog A (B.P.A.); blood pressure of dog B (B.P.B.); lateral pressure of the perfused hind leg of dog B (L.B.).

At 1, intravenous injection into spinal dog of 0.4 mgm. adrenaline.

Note reflex vasodilatation in the perfused leg of dog B in response to arterial hypertension in spinal dog B.

Time in intervals of 3 seconds.

pathectomized dog, from whom the two paravertebral chains had been removed several months previously. In this experiment, the spleen of dog B (the totally sympathectomized dog) was perfused by dog A, and

was in connection with its owner (dog B) only by means of its extrinsic splenic nerves. It was also of interest in this experiment that electrical stimulation of the central end of the vagus nerve did not cause any constriction of the perfused spleen, nor any elevation of the general blood pressure.

The observation of the absence, in the totally sympathectomized dog, of the vasomotor reflexes normally induced by blood pressure changes in the carotid sinus is confirmatory of the work of Bacq, Brouha and Heymans (4) on this point. The absence of arterial hypertension on stimulation of the central end of the vagus nerve in the dog, totally sympathectomized for several months, is in accord with the similar observation of Heymans, Bouckaert and Jongbloed (5) on the acutely sympathectomized dog.

DISCUSSION. These experiments have demonstrated that in the dog deprived of both vagi and of the vasosensitive zones in the aortic arch and carotid sinuses, increase and decrease of the general blood pressure cause, respectively, vasodilatation and vasoconstriction in the splanchnic circulation (spleen) and in the periphery (leg). These vasomotor reactions are not of direct central origin. Nor are they of reflex origin from the lungs (6), since section of both vagi does not interfere with the demonstration of the vasomotor reactions. These reactions are still present in the spinal dog, but disappear completely after the acute destruction of the cord in the spinal dog.

These vasomotor reflexes caused by variations in the blood pressure in the spinal dog are, in great part, spinal reflexes originating in vascular zones sensitive to pressure, and located in the area supplied by the celiac and superior mesenteric arteries. The origin of these spinal vasomotor reflexes might correspond to the reflex mesenteric sensitivity observed by Gammon and Bronk (7), and located by those authors in the Pacinian corpuscles, although their results are not compatible with ours. In a series of experiments, which gave results which those authors regard as suggestive rather than conclusive, Gammon and Bronk observed reflex vasoconstriction in response to distention of the mesenteric vessels, while we obtained reflex vasodilatation following an increase of pressure in the splanchnic circulation, and a reflex vasoconstriction following a decrease of pressure in this circulation.

These spinal vasomotor reflexes which adapt vascular tone to the general blood pressure, however, do not appear to play an important part in the automatic and proprioceptive regulation of the general blood pressure, as do the vasosensitive zones in the aortic arch and the carotid sinuses. In fact, occlusion of the celiac and superior mesenteric arteries, that is, hypotension in the splanchnic organs, does not cause a reflex vasomotor reaction sufficient to provoke any augmentation of the general blood pressure.

It is probable that the spinal vasomotor reflexes under discussion play a rôle in the distribution of blood in the deep abdominal circulation and in the periphery. These reflexes may conceivably have a more far-reaching influence under certain special conditions, and in a minor and accessory manner, may join with the vascular zones in the aortic arch and the carotid sinuses in the regulation of the circulation and the general blood pressure.

SUMMARY

1. In the dog deprived of the vascular zones in the aortic arch and the carotid sinuses, increase and decrease of the general blood pressure still cause, respectively, vasodilatation and vasoconstriction in the splanchnic circulation (spleen) and in the peripheral circulation (leg). These vasomotor reactions persist after section of both vagus nerves.

2. In the spinal dog, after complete section of the neck, increase and decrease of the general blood pressure cause, respectively, vasodilatation and vasoconstriction in the splanchnic circulation (spleen) and in the peripheral circulation (leg). These vasomotor reactions disappear after the acute destruction of the spinal cord in the spinal dog.

3. The vasomotor reactions caused in a spinal dog by variations in general blood pressure are vasomotor reflexes produced, for the most part, by variations in the blood pressure in the organs supplied by the celiac and superior mesenteric arteries. Other vascular areas (thoracic, peripheral ?) also possess reflex sensitivity to endovascular pressure.

4. The spinal vasomotor reflexes induced by alterations in the general blood pressure, as well as the carotid sinus and aortic vasomotor reflexes and the arterial hypertension and reflex vasoconstriction normally induced by electrical stimulation of the central end of the vagus nerve, are absent in the totally sympathectomized dog.

5. The physiological rôle of spinal vasomotor reflexes is discussed with special reference to those reflexes brought about by alterations in the blood pressure in the abdominal circulation.

REFERENCES

- (1) HEYMANS, C. *Rev. Belge sc. med.* 1: 6, 7, 1929.
- (2) HEYMANS C., J. J. BOUCKAERT AND P. REGNIERS. *Le sinus carotidien et la zone homologue cardio-aortique.* G. Doin and Co., Paris, 1933.
- (3) NOWAK, S. J. G. AND A. SAMAN. *Arch. Int. de Pharm. et de Therap.* 51: 463, 1935.
- (4) BACQ, Z. M., L. BROUHA AND C. HEYMANS. *Arch. Int. de Pharm. et de Therap.* 48: 429, 1934.
- (5) HEYMANS, C., J. J. BOUCKAERT AND J. JONGBLOED. *Arch. Int. de Pharm. et de Therap.* 53: 265, 1936.
- (6) SCHWIEGK, H. *Pflügers Arch.* 236: 206, 1935.
- (7) GAMMON, G. D. AND D. W. BRONK. *This Journal* 114: 77, 1935.

A STUDY OF "SIMPLE DISUSE ATROPHY" IN THE MONKEY¹

HERMAN CHOR AND RALPH E. DOLKART

*From the Department of Nervous and Mental Diseases and the Department of Chemistry,
Norwestern University Medical School*

Received for publication July 1, 1936

Other than in immobilization as occurs, for example, in the treatment of fractures, there are very few conditions which cause simple disuse atrophy of skeletal muscles. Immobilization experiments have been carried out by Froboese (1), Legg (2), Thompson (3) and Lippman and Selig (4), but their results are not in agreement. Differences in technique are, no doubt, responsible for many of the discrepancies. The present study was undertaken in an attempt to clarify this problem.

METHOD. Six young macacus rhesus monkeys were employed in the experiment. By applying a body and leg cast, an attempt was made to reduce the activity of the gastrocnemius-soleus muscles to a minimum, although it was realized, of course, that muscles so immobilized are still subject to "static" activity due to "stretch" and other tonic reflexes, which influences can only be abolished by nerve section.

Before applying the plaster, the extremity was first placed in a stockinette sleeve extending distally beyond the toes and proximally high up over the abdomen. The extremity was then wrapped very thoroughly with cotton batting to prevent pressure sequellae. Preliminary experiments showed that ischemic paralysis and pressure sores would be produced very easily by improper applications of the cast, and that the success of the experiment depended primarily upon the avoidance of such complications. In applying the plaster bandages, the limb was held in a position of slight flexion at the knee, in order to obtain maximum relaxation of the gastrocnemius soleus muscles and to avoid stretching. At designated periods of one, two, three, four, six, and ten weeks, respectively, the casts were removed. Using aseptic technique, the gastrocnemius-soleus muscles were dissected from their proximal and distal attachments.

Immediately following the excision, the muscle group was weighed and then bisected longitudinally. One portion was used for histologic study and the other for chemical analyses. For histologic studies, the sections

¹ This work was carried on with the assistance of a grant from the Council on Physical Therapy of the American Medical Association.

were stained by the hematoxylin and eosin, Van Gieson,— and a modified Ranson pyridine silver—methods.

In chemical analyses, water content was determined by desiccation of the muscle over sulphuric acid under reduced vapor pressure. Total nitrogen was determined by the Kjeldahl method and the protein content calculated.

In several preliminary experiments, the atrophied muscles were tested for threshold responses to faradic and galvanic stimulation and compared with the controls.

RESULTS. Within one week after immobilization there was gross evidence of a decrease in muscle bulk. This was verified by weighing, which revealed a loss of 4.9 per cent, as compared to the control side. In the

TABLE 1
Atrophy of simple disuse

	ONE WEEK		TWO WEEKS		THREE WEEKS		FOUR WEEKS		SIX WEEKS		TEN WEEKS	
	Left Control	Right Atrophy	Left Control	Right Atrophy	Left Control	Right Atrophy	Left Control	Right Atrophy	Left Control	Right Atrophy	Left Control	Right Atrophy
Wt. specimen on removal...	12.28	11.67	12.19	10.62	13.59	11.45	21.48	15.65	20.43	14.92	14.44	9.77
Weight difference.....	0.61		1.57		2.14		5.83		5.51		4.71	
Per cent wt. loss.....	4.9%		12.8%		15.7%		27.1%		29.8%		32.5%	
Wt. $\frac{1}{2}$ to be desiccated.....	5.53	5.37	6.25	5.15	7.61	6.16	11.63	8.74	11.78	6.56	6.80	4.48
Constant wt. after prolonged desiccation.....	1.46	1.38	1.57	1.35	1.88	1.49	2.90	2.24	3.04	1.68	1.73	1.25
Water content in gms.....	4.06	3.99	4.67	3.79	5.73	4.67	8.73	6.50	8.74	4.88	5.17	3.23
Per cent water.....	73.5%	74.2%	74.8%	73.6%	75.3%	75.8%	75.0%	74.3%	74.0%	74.3%	74.5%	72.1%
Total nitrogen.....	0.196	0.195	0.216	0.185	0.263	0.208	0.400	0.278	0.402	0.246	0.232	0.151
Protein N \times 6.25	1.225	1.217	1.351	1.156	1.645	1.299	2.502	1.742	2.511	1.537	1.449	0.943
Per cent protein.....	22.0%	22.5%	21.6%	22.5%	21.6%	21.1%	20.9%	21.2%	21.4%	21.9%	21.3%	21.0%
Specimen number.....	1	2	3	4	5	6	7	8	9	10	11	12

other specimens subjected to longer periods of inactivity (2, 3, 4, 6 and 10 weeks, respectively), the atrophy was found to be increased accordingly, as indicated in table 1.

Grossly, the atrophied muscles appeared somewhat paler than those of the control side. Microscopically, the muscle bundles were definitely smaller in diameter. The individual muscle fibers were narrower in cross-section and in longitudinal section showed prominent cross-striations, the Q bands appearing denser than normal. In cross-section, the fibers appeared more homogeneous, and Cohnheim's zones were less prominent, implying a decrease in the amount of sarcoplasm. Longitudinal striations were not observed. The sarcolemmal and muscle nuclei were not obviously increased in number nor altered in size, and there was no evidence of mito-

sis. The connective tissue elements were not increased. There was no change in the intra-muscular blood vessels. The intramuscular nerves and nerve endings were intact and normal in appearance, as demonstrated by the silver stain.

Chemical studies showed that the proportions of water and protein remained essentially the same as in normal muscle. For example, in the ten-week specimens, despite a 32.5 per cent weight difference between the atrophied and the control muscles, the variation in protein content between the two was but 0.30 per cent, and the difference in water content was too slight to have any significance.

Electrical stimulation of the atrophied muscles with faradic and galvanic current gave prompt responses similar to those obtained in normal skeletal muscle.

DISCUSSION. Formerly, the atrophy resulting from lesions of the nervous system, as well as in the arthritides in which activity is limited, has been attributed to disuse. This concept of disuse atrophy, however, is not strictly correct and needs to be clarified.

By simple atrophy of disuse is meant that wasting of muscle tissue which results solely from a curtailment of its specific function, namely, contraction, and without any accompanying disturbance of its nerve or blood supply. The atrophy resulting from disuse was found to be a very slow process. As such, it differs greatly from muscle atrophy resulting from lower motor neuron lesions. Computation of weight loss by comparing the inactive muscles with those of the opposite extremity, is subject to error. It may be pointed out that because of the limitation of activity of the one extremity, there was an increased use imposed upon the opposite one, which might result in some hypertrophy. Such a criticism seems quite justified. On the other hand, when one compares the general behavior of the animals used with that of the untreated animals, it is quite evident that the former group are less active than normally. Another fact to be considered is the normal variation in weight of the same muscle groups of opposite extremities. Studies by Lipschutz and Audova (5) on normal rabbits indicate that this variation is slight.

Histologic studies established the fact that this type of atrophy is associated with very simple structural changes. The loss in muscle bulk can apparently be accounted for on the basis of a diminished quantity of sarcoplasm in the individual muscle fibers. It is quite likely that slight changes also occur in the myofibrillar elements. If so, the changes are not revealed by the usual staining methods. The atrophied muscle fibers appeared to be packed more closely together, so that in a microscopic field of a definite area, a larger number of fibers was seen than in a corresponding area of normal muscle. There was no evidence of degeneration of the

muscle fibers, nor were there any of the usual features indicating an attempt at regeneration so characteristic of neurogenic atrophies.

Chemical analyses for water and nitrogen showed no significant change in water content between the atrophic and the normal muscle. The differences were small and occurred in both directions. This makes it highly improbable that there was any change in the degree of hydration of the protein. The quantitative decrease in protein content of this type of atrophy is associated with a decrease in the weight of the total muscle bulk, so that the relative proportion remains the same as that of normal muscle.

Physiologic tests likewise gave evidence of the absence of any degenerative process. Faradic and galvanic stimulation produced responses similar to those obtained in normal muscle.

Thompson, using rabbits, reported degeneration of muscle fibers with fibrosis, after six weeks' immobilization. He commented, however, upon the occurrence of circulatory and pressure complications in his experiment. This, no doubt, also accounted for the very rapid wasting which occurred. Froeboese, likewise, obtained degeneration of muscle fibers and replacement with fibrous tissue. Edema and pressure from the cast, no doubt, were responsible for these changes. It has been demonstrated by the present study that when such complications are avoided, the inactivity of muscle, such as is obtained by immobilization, does not give rise to any degeneration of muscle tissue.

Davenport and Ranson (6) studied the changes in skeletal muscle following tenotomy. After a period of from five to eight days, contracture occurred with a 20 per cent loss of weight. There was no degeneration of muscle fibers, nor increase in nuclei. They found an increase in the diameter of the fibers, more pronounced longitudinal striations, and blurred and wavy cross striations, which they considered characteristic of myostatic contracture. Such muscles, however, have been deprived of their "stretch reflex," and, hence, an additional factor has been added to that of simple inactivity. This, no doubt, accounts for the very rapid rate of atrophy resembling that which results following nerve section.

Lippman and Selig (4) found only a slight amount of muscle atrophy following fixation of the limb of the rabbit. They stated that such atrophy is not appreciable before the lapse of at least a month. Our results, however, imply a more rapid rate of atrophy, which corresponds to that commonly observed clinically.

In order to ascertain the status of the anterior horn cells which subserve the skeletal musculature subjected to inactivity, one monkey was treated as follows:

The left upper extremity was immobilized in a plaster of Paris cast for a period of one year. During this period the cast was removed at three-

month intervals to determine the state of the immobilized extremity, particularly to insure the avoidance of ischemic and pressure complications. Histologic examination of the atrophied muscles showed simple changes similar to those reported above. The cervical enlargement of the spinal cord and the peripheral nerves derived therefrom were examined. Employing hematoxylin and eosin and the Davenport silver stain, no difference could be demonstrated between cells of the right and left anterior cornu of the spinal cord. The peripheral nerves were likewise normal in appearance.

The factor of disuse has been mentioned by some in explanation of the changes observed in the anterior horn cells subserving extremities which have been amputated (Spatz, 7). These changes, designated as "axonal chromatolysis," have been considered retrograde and consist of a displacement of the nucleus to the axon hillock and a clumping of the Nissl bodies in that locality with a paling of the remaining cytoplasm. Other authors attribute these changes to injury to the axons.

The results of our experiment indicate that such muscular inactivity as is obtained by immobilization does not lead to demonstrable changes in the anterior horn cells of origin of the respective nerve supply. This is further supported by the lack of any changes in the intramuscular motor nerve endings.

CONCLUSIONS

1. Disuse atrophy is a distinct entity and is simple in character, as revealed by histologic findings.
2. Disuse atrophy consists primarily of a uniform reduction of the bulk of each muscle cell, especially of the sarcoplasm. It is not attended by any evidence of degeneration or attempts at regeneration. Irritability to electrical stimuli remains unaffected.
3. There is no alteration in the proportions of water and nitrogen content.
4. Simple disuse is not associated with any demonstrable changes in the anterior horn cells of origin of the respective nerve supply, and the atrophy of the peripheral musculature due to disuse does not result in such changes.

REFERENCES

- (1) FROBOESE, C. *Mitt. a. d. Grenz. ges. d. Med. u. Chir.* **35**: 683, 1922.
- (2) LEGG, A. T. *Am. J. Orth. Surg.* **6**: 81, 1908.
- (3) THOMPSON, T. C. *J. Bone and Joint Surg.* **16**: 569, 1934.
- (4) LIPPMAN, R. T. AND S. SELIG. *Surg., Gynec. and Obstet.* **47**: 512, 1928.
- (5) LIPSCHUTZ, A. AND A. AUDOVA. *J. Physiol.* **55**: 300, 1921.
- (6) DAVENPORT, H. A. AND S. W. RANSON. *Arch. Surgery* **21**: 995, 1930.
- (7) SPATZ, H. *Ztschr. f. d. ges. Neurol. u. Psychiat.* **58**: 327, 1920.

THE EFFECT OF METHYLENE BLUE, CYSTINE AND CYSTEINE ON THE METABOLISM OF THE INTACT ANIMAL¹

WALTER GOLDFARB, JOSEPH F. FAZEKAS AND HAROLD E. HIMWICH

*From the Laboratories of Physiology, Albany Medical College, Albany, New York, and
Yale University School of Medicine, New Haven, Connecticut*

Received for publication July 10, 1936

It is well known that the foodstuffs which may be oxidized readily by the body are very resistant to oxidation in the test tube; hence the hypothesis arose that either the oxygen, or the food substrate, or both, were activated by enzymes in the body tissues. Warburg (1928, 1930) emphasized the activation of oxygen, and noted especially the significance of the catalysis by heavy metals; Wieland (1922, 1931), Thunberg (1930) and others presented evidence of the part played by the activation of the hydrogen atoms of the substrate. Later research on these problems demonstrated that both mechanisms were of importance in the oxidation of foodstuffs (Keilin, 1929, 1930; Barron and Hastings, 1933).

Other investigators have demonstrated that the addition of redox substances to tissues increased the rate of dehydrogenation of various substrates (Barron and Harrop, 1928; Barron, 1930; Wendel, 1933). The fact that the velocity of this reaction was increased by the addition of a hydrogen transporter indicated that more hydrogen might have been activated by the tissue dehydrogenases than could be taken up by the hydrogen transporters already present in the system. We have therefore investigated the effects of the addition of exogenous redox substances both in intact animals and isolated tissues.

EXPERIMENTS ON THE INTACT ORGANISM. The first group of observations were obtained on normal rats. Analyses of O₂ and CO₂ exchange were made in the Haldane (1892) open circuit respiratory chamber. The rats were usually fasted for 24 hours, and a determination of the respiratory exchange made. They were then injected with 0.5 cc. of 0.01 M methylene blue per 100 grams of body weight. The O₂ consumption and CO₂ production were subsequently determined during successive periods for 45 hours. The data obtained on 8 rats are presented in table 1.

It may be observed that the injection of methylene blue resulted in a depression of the R.Q. to levels as low as 0.55 in one case, and below 0.70 in almost all cases.

¹ Preliminary Reports, Proc. Soc. Exper. Biol. and Med. 30:906, 1933; This Journal 113: 51, 1935.

This regular depression of the R.Q. followed by a rise is not to be observed in the control animals which were also fasted for twenty-four hours before observations were made but were not injected with methylene blue.

TABLE 1
Effect of methylene blue on the metabolism of normal rats

PERIODS	ANIMAL 1	ANIMAL 2	ANIMAL 3	ANIMAL 4	ANIMAL 5	ANIMAL 6	ANIMAL 7	ANIMAL 8
<i>hours</i>								
Foreperiod	0.69	0.76	0.73	0.72	0.72	0.77	0.70	0.90
0-5	0.63	0.67	0.68	0.69	0.57	0.68	0.64	0.82
5-10	0.67		0.72	0.55	0.59	0.71	0.64	0.73
10-15	0.73	0.78	0.73			0.70	0.66	
15-20	0.80	0.84	0.72				0.73	0.69
20-25	0.82		0.72	0.60	0.61	0.69		0.84
25-30	0.75	0.70		0.79	0.83	0.81	0.78	0.71
30-35	0.77	0.72		0.75	0.82	0.73	0.74	
35-40	0.76		0.71					0.74
40-45	0.74		0.70	0.73	0.68		0.71	
Total R.Q.	0.737	0.741	0.711	0.670	0.683	0.711	0.710	

TABLE 1A
Control observations on fasted rats

PERIODS	ANIMAL 1	ANIMAL 2	ANIMAL 3
<i>hours</i>			
0-5	0.72	0.67	0.73
5-10	0.73	0.69	0.74
10-15	0.73	0.71	0.74
15-20	0.74	0.72	0.75
20-25	0.74	0.74	0.76
25-30	0.73	0.72	0.75
30-35	0.72	0.69	0.73
35-40	0.73	0.70	0.74
40-45	0.76	0.74	0.76
45-50	0.74	0.72	0.76
50-55	0.73	0.68	0.76
55-60	0.73		0.74
60-65	0.71		0.70
65-70	0.70		0.66
Average	0.729	0.707	0.737

The R.Qs. of table 1A reveal the small variations from the level of fat combustion.

On the other hand, in a number of instances, namely, experiments 1, 4, and 5 of table 1, the quotients were lower than could be explained by any

known type of physiological oxidation. This decrease in the quotient lasted in most cases from 10 to 20 hours. It was followed by a secondary rise which usually exceeded the quotient of the foreperiod. The R.Q. then fell gradually to the postabsorptive level. In those instances in which the R.Q. of the foreperiod approximated that attained during fasting, the quotient for the entire experiment was also found to be close to 0.70. The average quotient of these experiments was 0.709 ± 0.009 .

The fact that the R.Q. of the entire experiment approximated that of the fasted animal indicated that the metabolic energy was obtained from a food mixture similar to that oxidized during starvation. Such a food mixture would consist of fat, protein, and minimal amounts of carbohydrate or lactic acid. It was of interest, however, to investigate the intermediary

TABLE 2

Effect of methylene blue on the metabolism of phlorhizinized rats

PERIODS	ANIMAL 1	ANIMAL 2	ANIMAL 3	ANIMAL 4	ANIMAL 5
<i>hours</i>					
Foreperiod	0.69	0.72	0.73	0.72	0.68
0-10	0.63	0.62	0.68	0.63	0.63
10-20	0.71	0.66	0.65	0.55	0.58
20-30	0.65	0.66	0.54	0.60	0.60
30-40	0.64	0.71	0.56	0.57	0.53
40-50	0.74	0.78	0.65	0.68	0.68
50-60	0.83	0.83	1.10	0.88	1.05
60-70	0.82	0.80	1.13	0.88	1.12
70-80	0.70	0.67	0.99	0.86	1.05
80-90			0.84	0.86	0.94
90-100			0.71	0.68	0.69
Total R.Q.	0.702	0.705	0.730	0.680	0.710

processes involved which resulted in such peculiar variations of the quotient. The first possibility that suggested itself was that methylene blue might cause a conversion of fat to carbohydrate, and therefore a depression of the R.Q. below 0.70. The secondary rise of the quotient seemed to follow the appearance of the methylene blue in the urine, and may have resulted from the oxidation of the newly formed carbohydrate.

The net result of these intermediary reactions, namely, the conversion of fat to carbohydrate plus the oxidation of the carbohydrate, would yield a R.Q. of 0.707 since the substrate oxidized was fat. To test this possibility a similar group of experiments were performed on phlorhizinized rats. In the phlorhizinized rat the renal threshold for glucose is reduced. Any carbohydrate formed from fat would be excreted and the secondary rise of the R.Q. should, therefore, fail to appear. The rats were fasted for three

days and injected with 20 mgm. of phlorhizin per 100 grams of body weight per day. We have previously noted a marked excretion of glucose in rats injected with a slightly smaller dose of phlorhizin (Goldfarb, Barker and Himwich, 1934). Metabolism determinations were made before and after the injection of 0.5 cc. of 0.01 M methylene blue per 100 grams of body weight. The results are summarized in table 2.

It may be seen that the data obtained closely resembled those of the animals receiving no phlorhizin. The original R.Q. was close to that characteristic of the post-absorptive condition. The first change due to the injection of methylene blue was a depression of the quotient below the normal physiological range. This was followed by a secondary rise which exceeded the original quotient in all cases. The R.Q. then gradually returned to the fasting level. The quotients obtained for the entire experiment varied from 0.670 to 0.730 with an average of 0.705 ± 0.006 . The secondary increase of the R.Q. could not be explained by the oxidation of glucose since this substance is readily excreted by the phlorhizinized animal. It therefore seems probable that there was no conversion of fat to carbohydrate.

A second hypothesis suggested which might account for these variations of the R.Q. depends on the fact that methylene blue is an oxidation-reduction substance. We therefore investigated the effects of two other substances which are known to function as the effective portion of a redox system in the body (Hopkins, 1921), namely, cystine \rightleftharpoons cysteine. The method was the same as that used above. Five rats were injected with 1 cc. of 0.01 M cysteine per 100 grams of weight, and 5 others received 0.5 cc. of 0.01 M cystine per 100 grams of weight. The data are presented in table 3.

It was again observed that the quotients were depressed in the early hours, subsequently rose above the original R.Q. and then gradually returned to the fasting level. In 9 of these experiments in which the original quotient was approximately that obtained under fasting conditions, the quotient for the entire experiment approached 0.70. The average quotient in these animals was 0.709 ± 0.006 .

The changes in the R.Q. following the injection of methylene blue, cystine or cysteine may have been due either to changes in the O_2 consumption, or CO_2 production, or both. In the present experiments the variations were found to result more from changes in the O_2 than the CO_2 . The relationship between the R.Q. and the O_2 consumption (table 4) demonstrates that the low R.Qs. are associated with high O_2 consumption, and vice versa.

DISCUSSION. In the present work we have investigated the effects of the addition of hydrogen transporters to the oxidative systems in the intact animal. The injection of methylene blue, cystine or cysteine into

rats has been found to produce similar changes in respiratory metabolism. These substances first caused a fall of the R.Q. below the levels which might be expected from any known form of physiological oxidation. Subse-

TABLE 3

Effect of cysteine and cystine on the metabolism of normal rats

1 cc. 0.01 M CYSTEINE PER 100 GRAMS						0.5 cc. 0.01 M CYSTEINE PER 100 GRAMS					
Periods	Animal 1	Animal 2	Animal 3	Animal 4	Animal 5	Periods	Animal 1	Animal 2	Animal 3	Animal 4	Animal 5
<i>hours</i>											
Foreperiod	0.72	0.74	0.74	0.73	0.79	Foreperiod	0.77	0.67	0.71	0.76	0.68
0-11	0.68	0.73	0.73	0.64	0.76	0-5			0.68	0.66	0.64
11-24	0.69	0.71			0.74	5-10	0.75	0.65	0.71	0.69	0.69
24-30	0.64	0.69				10-13	0.73	0.62			
30-37	0.67	0.68	0.65	0.61	0.75	13-24	0.63	0.66	0.67	0.69	0.71
37-48	0.63	0.63	0.73	0.72	0.75	24-30	0.82	0.72	0.71	0.72	0.71
48-55	0.67	0.73			0.80	30-33	0.74	0.85			
55-61			0.79	0.72		33-38	0.74	0.80	0.73	0.73	0.68
61-72	0.77	0.81	0.93	0.75	0.77	38-47	0.73	0.80	0.69	0.72	0.69
72-84	0.87	0.81	0.72	0.76	0.73	47-52		0.73			
84-95	0.79	0.77		0.80	0.72	52-59			0.72	0.71	0.70
95-105	0.72	0.77		0.69							
105-120	0.66	0.69									
Average...	0.707	0.730	0.727	0.700			0.720	0.712	0.696	0.701	0.694

TABLE 4

Relation between R.Q. and O₂ consumption

R.Q.	OXYGEN CONSUMPTION		
	+	0	-
0.51-0.60	12	0	0
0.61-0.65	19	2	1
0.66-0.70	27	3	19
0.71-0.75	26	2	27
0.76-0.80	4	5	12
0.81-0.90	0	0	18
0.91-1.0	0	0	2
1.01-1.10	0	0	4

Coefficient correlation—(-0.646 ± 0.027).

* +: higher than average for entire experiment. 0: equal to average for entire experiment. -: less than average for entire experiment.

quently the quotients rose above those of the foreperiod, and then gradually fell to the fasting level. Hawley, Johnson and Murlin (1933) have calculated the total fall in R.Q. which might result from an abnormal protein

metabolism, plus the conversion of glycerol to fat, and the development of ketosis. The combined correction for these three processes was 0.04. Since the fall in the quotient in the present experiments far exceeded this value, some other explanation must be sought.

The changes of the R.Q. were not due to the conversion of fat to carbohydrate since they occurred in the phlorhizinized animal. Such a conclusion is in harmony with the great mass of evidence which has failed to demonstrate the conversion of fat to carbohydrate in the mammalian organism (Lusk, 1928; Dann, 1933).

It was noted that in the experiments in which the R.Q. of the foreperiod approached 0.70, the quotients for the entire experiment also approximated 0.70. This indicated that the energy for metabolism during the entire experiment was derived from the complete oxidation of a food mixture which resembled that of fasting conditions. The mechanism of oxidation, however, evidently took place in two steps, the first of which resulted in a depression of the R.Q., while the second phase caused a rise of the quotient. In order to explain these results, it is suggested that the addition of the oxidation-reduction system facilitated the removal of H_2 from some substrate which was activated by tissue dehydrogenases. The reduced redox substance might then be readily oxidized by O_2 to yield H_2O plus the dehydrogenated form of the added substance. This reaction would result in the consumption of O_2 without the simultaneous production of CO_2 and the R.Q. would fall.

In the experiments with methylene blue the dye was observed in the urine during the first 24 hours. The secondary rise of the quotient occurred after the excretion of the dye. We have no data on the fate of the injected cystine or cysteine, but some estimate of the rate of excretion may be obtained from the work of Lewis and his collaborators. Lewis and Root (1922) recovered 73 per cent of the S of ingested cystine in the first 24 hour urine specimen. Lewis et al. (1924) also found that the administration of phenyluraminocystine to rabbits resulted in the conversion of an average of 50 per cent of the recovered compound into the cysteine derivative. It therefore seemed probable that the onset of secondary rise of the quotient in our experiments corresponded approximately with the disappearance of the hydrogen transporters from the body. If the fall of the R.Q. following the injection of a hydrogen transporting system is due to an increased velocity of dehydrogenation of some food substrate, the removal of the stimulus ought to result in a cessation of the process with a return of the quotient to the normal fasting level. However, instead of returning to post-absorptive values, the quotient reached far higher ones. This secondary rise of the R.Q. may be due to one or both of two intermediary reactions; the carbon rich residues might be oxidized and yield a high quotient, or the dehydrogenated compounds might be reduced

by accepting hydrogen. Either of these processes, or both combined, would result in the rise of the R.Q. observed. Michaelis and Smythe (1936) recently suggested that methylene blue inactivates carboxylase. Such an effect of methylene blue *in vivo* would diminish the formation of carbon dioxide and may explain in part the method of production of the carbon rich residues.

SUMMARY AND CONCLUSIONS

The respiratory exchange of fasted rats was studied after the injection of methylene blue, cysteine and cystine. The changes of R.Q. with each of these substances were similar. The R.Q. was depressed during the early hours, subsequently rose above the original quotient, and then returned to the post-absorptive levels. The results are best explicable on the basis of an increased velocity of dehydrogenation of some food substrate during the early hours of the experiment with the resulting formation of water. The secondary rise of the respiratory quotients was due either to the complete oxidation of the carbon rich residues, or the reconversion to their original form.

REFERENCES

- BARRON, E. S. G. J. Exper. Med. **52**: 447, 1930.
BARRON, E. S. G. AND G. A. HARROP, JR. J. Biol. Chem. **79**: 65, 1928.
BARRON, E. S. G. AND A. B. HASTINGS. J. Biol. Chem. **100**: 155, 1933.
DANN, M. Yale J. Biol. and Med. **5**: 359, 1933.
GOLDFARB, W., S. B. BARKER AND H. E. HIMWICH. J. Biol. Chem. **105**: 283, 1934.
HALDANE, J. J. Physiol. **13**: 419, 1892.
HAWLEY, E. E., C. W. JOHNSON AND J. R. MURLIN. J. Nutrition **6**: 523, 1933.
HOPKINS, F. G. J. Biochem. **15**: 286, 1921.
KEILIN, D. Proc. Roy. Soc. London **104B**: 206, 1929.
Proc. Roy. Soc. London **106B**: 418, 1930.
LEWIS, H. B. AND L. E. ROOT. J. Biol. Chem. **50**: 303, 1922.
LEWIS, H. B., H. UPDEGRAFF AND D. A. MCGINTY. J. Biol. Chem. **59**: 59, 1924.
LUSK, G. The science of nutrition. 4th ed., 1928.
MICHAELIS, L. AND C. V. SMYTHE. J. Biol. Chem. **113**: 717, 1936.
THUNBERG, T. Quart. Rev. Biol. **5**: 318, 1930.
WARBURG, O. Ueber die Katalytischen Wirkungen der lebendigen Substanz.
Springer, Berlin, 1928.
Bull. Johns Hopkins Hosp. **46**: 41, 1930.
WENDEL, W. B. J. Biol. Chem. **102**: 373, 385, 1933.
WIELAND, H. Ergebn. d. Physiol. **20**: 477, 1922.
Ztschr. f. angew. Chem. **44**: 579, 1931.

STRYCHNINE AND THE CHRONAXIE

P. K. KNOEFEL

From the Pharmacological Laboratory of the University of California Medical School, San Francisco, and the Department of Physiology and Pharmacology, University of Louisville School of Medicine

• Received for publication July 13, 1936

The studies of the Lapicques on the disturbance of conduction between motor nerve and striated muscle, the phenomenon of curarization, included the block produced by strychnine (1). They found that administration of strychnine to the whole frog or application to the isolated nerve and muscle while not changing the chronaxie of muscle reduced that of the nerve, and they interpreted this as an "acceleration of the process of excitation." This phenomenon they used in support of an hypothesis of isochronism of muscle and nerve, and a general theory of curarization, as it supplied a method of production of heterochronism alternative to that which they had found with curare. In addition, they stated that when block occurred with strychnine the chronaxie of nerve had fallen to one-half its original value, thus the degree of heterochronism, a 1 to 2 ratio, was the same as seen with curare.

These results were confirmed by Bremer and Rylant (2) and extended by them in the development of a theory of the mode of action of strychnine on the central nervous system. The beliefs of the Lapicques have recently been reaffirmed (3). Still, it appeared that the action of strychnine would bear reëxamination.

EXPERIMENTAL. The procedure was identical with that used in a study of the action of narcotics on nerve (4). The cord-sciatic-gastrocnemius preparation of the Louisiana bull-frog was mounted in a paraffin chamber with two pairs of silver-silver chloride electrodes in Ringer's solution (NaCl 6.5, KCl 0.14, CaCl_2 0.12, NaHCO_3 0.2, NaH_2PO_4 0.01 gram per liter) and left for at least two hours before applying the drug, strychnine sulfate in this saline solution. The voltage-capacity relationship was determined with a condenser apparatus as used previously (4). Eighteen experiments were done, with application of strychnine either to the nerve at the cathode of the peripheral electrodes, or to this point and the muscle. The results of a typical experiment are shown in figure 1. The change in excitability, reversed by removal of the strychnine, was seen only at the peripheral pair of electrodes at which point the drug was

applied to the nerve. As this did occur without change at the central electrodes, it was an alteration in excitability of the fibers originally responding.

With concentrations below 0.025 per cent there was no discernible change in the excitability of the nerve, but at this concentration the typical effect as shown in figure 1 appeared and increased with increase in concentration until at 0.25 per cent applied to the nerve alone block occasionally occurred within three hours. With application to the muscle, a concentration of 0.05 per cent produced loss of indirect excitability within four hours; sooner with higher concentrations. Response of the muscle to direct stimulation was not lost with a concentration of 0.25 per cent.

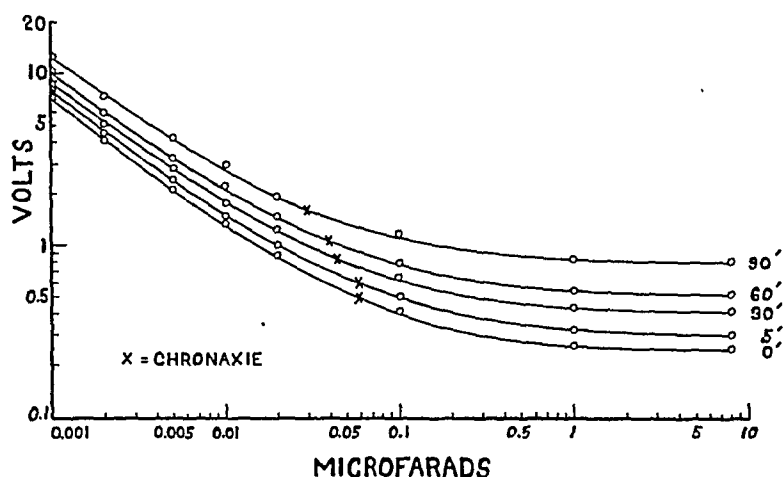


Fig. 1. (7/24/35) One-tenth per cent strychnine sulfate applied to muscle and to nerve at cathode of peripheral pair of electrodes. Determinations of excitability at 15, 45, 75, and 105 minutes are not shown. Loss of excitability at 120 minutes.

No attempt was made in these experiments to avoid the possible intervention of an *alpha* effect from the use of electrodes in a fluid bath (Lapicque); as in the previous study (4) no evidence was obtained of this dubious phenomenon having any influence on the results.

DISCUSSION. The action of strychnine on the nerve fiber is not one of augmentation of excitability, as was concluded by the Lapicques, for it is indistinguishable from that of typical narcotics. Although the chronaxie is reduced, the *temps utile* is lengthened, the rheobase much increased. If excitability is defined in terms of the quantity or the energy of the electrical stimulus, as for instance with the formula of Lassalle (5):

$$\text{Excitability} = \frac{1}{(\text{Rheobase})^2 \times \text{Chronaxie}}$$

the effect of strychnine is clearly seen to be one of reduction of excitability.

In general, the evidence of others indicates a purely depressant action of strychnine on peripheral nerve fibers. Biberfeld (6) found only depression, and Danilewsky and Perichanjanjz (7) claimed stimulation with low concentrations, but these early experiments were technically inadequate. Bronk (8) found chiefly a depressant action on sensory end organs in frog muscle and skin. Cowan and Ing (9) have shown that 0.01 molar strychnine hydrochloride (0.01 molar strychnine sulfate = 0.8 per cent) completely blocks the unmedullated nerve of *Maia squinado* in thirty minutes.

In the papers of the Lapiques, much is made of the fact that conduction between nerve and muscle failed when the chronaxie of the nerve had fallen to one-half its original value. This information is of questionable value as the excitability that is measured shortly before total failure of conduction is not that of the nerve fibers originally responsive to the minimal stimuli. This is shown by the rise in threshold that occurs at the central electrodes shortly before complete block takes place, indi-

TABLE 1

CONCENTRATION OF STRYCHNINE	TIME OF BLOCK	CHRONAXIE: PER CENT OF NORMAL AT TIME OF BLOCK
<i>per cent</i>	<i>minutes</i>	<i>per cent</i>
0.10	120	53
0.25	105	67
0.25	90	68
0.25	75	75
0.25	60	86
0.25	40	95

cating that the most sensitive fibers stimulated at first now fail to excite the muscle fibers they innervate. In these experiments no constancy of reduction in chronaxie at the time of block was observed. In table 1 is shown the percentage of normal to which the chronaxie had fallen at a time shortly preceding block, and the time at which block took place. The obvious relationship indicates that in the longer time required for the production of block, the greater has been the depression of the nerve at the stimulating electrodes. It would suggest that the diffusion of the agent into nerve is regular, that into muscle very irregular.

As strychnine can interrupt conduction when applied to the nerve fiber alone, it is likely that this process sufficiently accounts for the failure of indirect excitability of muscle. The difference between the concentration required to block the nerve trunk and that required to interrupt neuro-muscular conduction may be due to the generally assumed high susceptibility of the terminations of the nerve fibers.

It is a pleasure to acknowledge the criticisms and advice of Dr. Hallowell Davis and the loan of apparatus by Dr. J. M. D. Olmsted.

SUMMARY

The action of strychnine on the motor nerve fiber is purely one of depression. The fall in chronaxie which occurs does not mean an augmentation of excitability as the Lapiques believed, and their conclusion that strychnine interrupts conduction between nerve and muscle by producing a heterochronism is unjustified.

REFERENCES

- (1) LAPICQUE, M.: *Compt. rend. Soc. Biol.* 62: 1062, 1907.
LAPICQUE, L. AND M. LAPICQUE. *Ibid.* 65: 733, 1908; 74: 1012, 1913.
- (2) BREMER, F. AND P. RYLANT. *Compt. rend. Soc. Biol.* 91: 110, 1924; 92: 199, 1324, 1925.
- (3) LAPICQUE, M. *Compt. rend. Soc. Biol.* 111: 957, 1932.
LAPICQUE, L. *J. Physiol.* 81: 113, 1934.
- (4) KNOEFEL, P. K. *J. Pharmacol. Exper. Therap.* 55: 72, 1935.
- (5) LASSALLE, H. *Compt. rend. Soc. Biol.* 98: 273, 1928.
- (6) BIBERFELD. *Pflüger's Arch.* 83: 397, 1901.
- (7) DANILEWSKY, B. AND J. PERICHANJANZ. *Arch. f. Exper. Path. u. Pharmacol.* 105: 319, 1925.
- (8) BRONK, D. W. *J. Physiol.* 67: 17, 1929.
- (9) COWAN, S. L. AND H. R. ING. *J. Physiol.* 84: 90, 1935.

THE RELATIONSHIP OF THE SYNTHETIC MALE HORMONE, ANDROSTENDION, TO THE PROTEIN AND ENERGY METABOLISM OF CASTRATE DOGS, AND THE PROTEIN METABOLISM OF A NORMAL DOG¹

CHARLES D. KOCHAKIAN² AND JOHN R. MURLIN

From the Department of Vital Economics, University of Rochester, Rochester, N. Y.

Received for publication July 20, 1936

A previous report (Kochakian and Murlin, 1) from this laboratory has indicated that male hormone prepared from urine has no significant effect on the energy metabolism of castrate dogs whether administered in large single doses or in smaller doses over a period of time. A slight increase of about 10 per cent was noted in the case of a fat castrate dog after many repeated injections, but this effect was not considered very significant. In this same study it was noted that in every case there was a significant decrease in protein catabolism which was accounted for by a decrease in the urea fraction of the urine. The remaining constituents of urine and the nitrogen of the feces remained constant. It was also noted that during the repeated injections the decreased nitrogen catabolism attained a maximum within five days and further injections, or increasing the daily dosage, did not further increase the nitrogen retention, but only maintained it at the maximum.

In the meantime, the rapid advance in the chemistry of the male hormones has made available in pure crystalline form several interesting compounds related to the male hormones. Of these compounds, the synthetic male hormone, androstendion, was of particular interest to our study because of its midway position between the urinary products, which we have already studied, and the testicular product, testosterone, which we propose to study later. Androstendion possesses the α, β unsaturated ketone group of testosterone and the ketone group on the 5-membered ring of the urinary products, dehydroandrosterone and androsterone. Its potency on the capon's comb is equivalent to that of androsterone (2) (3) which is 3 to 4 times that of dehydroandrosterone (4), and only one-seventh

¹ This investigation was aided by a grant from the Committee on Scientific Research of the American Medical Association.

² This publication is taken from a thesis presented in partial fulfillment of the requirements for the degree Doctor of Philosophy, University of Rochester, June 1936.

that of testosterone (5), (6). Its action on the accessory sex organs lies midway between that of testosterone and androsterone and dehydroandrosterone (3). This unique biological and chemical midway position of androstendion, therefore, made an investigation of this compound of special interest to our studies.

Since the previous study showed that the same results were obtained on protein and energy metabolism by single large injections as by repeated smaller injections, which, however, produced a maximum in the protein retention within five days, it was felt that the purpose of this study could be most satisfactorily fulfilled by administering a large amount of hormone in three separate doses 24 hours apart. Thus the maximum effect on protein metabolism should be obtained and three instead of one experiment on energy metabolism made possible with more adequate recording of the same kind of results as are obtained by repeated injections. In order to eliminate the possibility that the decreased urea excretion might be due to retention of urea, the blood N.P.N. was determined. Also it seemed of interest to see if the rectal temperature of the dogs would be affected, since increase in body temperature is often associated with increased metabolism.

The retention of nitrogen noted in the previous report was attributed to regeneration of the accessory sex glands of the castrate animals. If this is the only use made of the nitrogen, then the fact noted that once a maximum retention of nitrogen was attained no further increase could be induced, either by further injections or by larger daily injections, would seem to indicate that a certain amount of hormone was required to maintain the physiological function of these glands at their maximum. It would follow, then, that a normal dog, producing his own male sex hormone to maintain the physiological functions of his accessory glands, would not react to the injections of male sex hormone as do the castrate dogs. Therefore, a normal dog was included in the protein metabolism phase of this study.

Preparation of androstendion. The preparation of androstendion was accomplished by means of the identical methods proposed almost simultaneously by Butenandt and Kudzuz (2) and Ruzicka and Wettstein (3). Two such preparations were made in our own laboratory for the purposes of this study. Preparation 1 was purified by repeated crystallization from dilute acetone. The final product, M.P. 164–5°C. (u.c.) was dissolved in olive oil so that 1 cc. of the oil solution was equivalent to 10 mgm. and was sterilized by passing through a Zeiss filter.³ Preparation 2 was purified by recrystallizing once from dilute acetone followed by high vacuum distilla-

³ We are indebted to Doctor Tittsler of the Department of Bacteriology for the sterilization.

tion⁴ (7) and repeated recrystallization of the sublimate from dilute acetone. The pure product M.P. 164.5–165.5°C. (u.c.) was dissolved in olive oil so that 1 cc. of solution was equivalent to 15 mgm. of androstendion and was sterilized by autoclaving at 15 pounds pressure for 20 minutes.

The solutions of androstendion were assayed by the method employed in this laboratory (1): One capon unit⁵ (C.U.) is the amount of hormone which, when injected daily on two successive days, will produce on the third or fourth day an average maximum increase of 3 to 4 mm. in length plus height (L + H) of the combs of at least three white leghorn capons. Nine capons were used for the assay and 1 mgm. of androstendion was found to be equivalent to 1.6–2.1 C.U.

Spectrophotometric studies⁶ gave a typical α,β unsaturated ketone curve with a maximum absorption at 2360 Å.

TABLE 1
Daily diet

	DOG 1, 15 KGM. DOG 6, 13.1 KGM.			DOG 2, 26 KGM.		
	Grams	Grams N	Calories	Grams	Grams N	Calories
Beef heart.....	175	5.48*	434	225	6.89*	563
Cracker meal.....	60	1.20	233	80	1.60	322
Lard.....	30		270	40		360
Cod liver oil.....	5		45	5		45
Bone ash.....	10			10		
Wesson's salt mixture.....	3			3		
Totals.....		6.63	982		8.49	1,290

* Varied with each batch of beef heart.

Description. Three mongrel dogs were used. Dogs 1 and 2 were castrates that had been used before and are described in the previous report (1). Dog 6 was a normal adult dog.

Diets. The dogs were maintained on a beef-heart-cracker meal diet similar to that previously (1) described. This diet has now been used for 2 years and found adequate in all respects. The details of the diet are given in table 1. Dogs 1 and 6 were placed on the same intake.

⁴ We wish to express our appreciation to Dr. Wm. M. Allen, Dr. W. H. Strain and Prof. W. R. Bloor for the use of their apparatus, and also to Doctor Allen for instruction in its proper use.

⁵ Henceforth we shall use the term capon unit (C.U.) instead of bird unit (B.U.) which we used in previous publications. (Proc. Soc. Exper. Biol. Med. 32: 1064, 1935; J. Nutrition 10: 437, 1935). This change is desirable because we feel that the new unit is more descriptive and specific than the old.

⁶ We are indebted to Dr. L. H. Stedman of the Department of Radiology for this study.

Procedure. The dogs were confined in metabolism cages and fed the prescribed diet at 5:30 p.m. Every other day catheterization was performed just before feeding. Two-day urine periods were employed except in cases noted in the tables. Total nitrogen of urine was determined by the usual Kjeldahl-Gunning method, urea plus ammonia by the Van Slyke-Cullen (8) aeration method and creatinine by the Folin (9) microchemical method. The ammonia was not determined separately since it had been shown to remain constant. The rest nitrogen was determined by difference. Creatine nitrogen was not determined, since in the previous report it was shown that any variations in creatine could not be considered significant because of the exogenous source of the creatine. Fecal nitrogen also was not determined because it had been shown to be constant.

The blood samples were taken from the jugular vein of dog 1 and the saphenous vein of dog 2. Potassium oxalate was used as the anti-coagulant. N.P.N. was determined on the Folin-Wu filtrate by Nesslerization (10). Blood urea was determined by the Leibhoff-Kahn (11) method.

The energy metabolism of dog 1 was determined by means of a Benedict closed circuit apparatus attached to an all-metal chamber, which was enclosed by a water jacket and contained thermometers reading to 0.02°C . at the air inlet, outlet and the top. The oxygen used was determined by passing through an accurately calibrated wet-test meter. Alcohol checks were made to ascertain the fitness of the apparatus. The average R.Q. of three such determinations was 0.663. Activity records were obtained by means of a kymograph.

"Helmet" for dog. The energy metabolism of dog 2 was obtained by the Tissot-Haldane method. An accurate spirometer was used for the collection of the expired air which was analyzed by means of a specially constructed modified Haldane apparatus of very good accuracy (12). The dog was under constant observation and respiration rates were repeatedly recorded. In the previous study the dog was connected to the spirometer by means of a face mask. Considerable difficulty was experienced in keeping the dog relaxed by means of this procedure. Therefore a Benedict type helmet (13) was constructed which proved very satisfactory. The bottom of an ether container 21 cm. by 16 cm. was removed and the ragged edges covered with adhesive tape. In the middle of the other end, the top, was soldered a 3 cm. x 4.5 cm. piece of brass tubing for an outlet to be connected to the flutter valve system of the spirometer. The dog's neck was shaved close up behind the ears and a dental dam neckpiece from a Benedict helmet was slipped over the dog's neck. The opening of this neck piece proved to be just the exact size for this dog. Then the can helmet was slipped over the dog's head and the dental dam was wrapped about the end of the can and held tightly there by means of a heavy elastic band. No sealing agent is needed about the dog's neck if the neck is kept well shaven and the proper size aperture is obtained in the dental dam.

TABLE 2
Blood chemistry
 Experiment 1, dog 2

DATE	N.P.N.	UREA N	REST N
1936	mgm. per cent	mgm. per cent	mgm. per cent
2/16	29.3	9.8	19.5
2/17	Injected 32-42 C.U. (20 mgm.)	Androstendion, 10:19 a.m.	
	28.1	11.4	16.7
2/18	Injected 32-42 C.U. (20 mgm.)	Androstendion, 9:24 a.m.	
	27.8	12.2	15.6
2/19	Injected 32-42 C.U. (20 mgm.)	Androstendion, 10:15 a.m.	
	27.0	9.8	17.2
2/20	26.7	11.6	15.1
2/21	27.2	11.0	16.2
2/22	28.7	12.3	16.4
2/23	27.5	11.3	15.2
2/24	29.1	12.1	17.0

TABLE 3
Summary of nitrogen metabolism
 Experiment 2, dog 2. Nitrogen intake, 8.49 grams N per day

DATE*	URINE CHEMISTRY—NITROGEN PER DAY				BLOOD CHEMISTRY		
	Total	Urea + NH ₃	Creatinine	Rest	N.P.N.	Urea N	Rest N
1936	grams	grams	grams	grams	mgm. per cent	mgm. per cent	mgm. per cent
3/6					27.8	12.2	15.6
3/7					26.9	11.7	15.2
3/8							
3/9					26.4	11.3	15.1
3/10							
3/11	7.67	5.67†	0.238	1.76	27.3	12.3	15.0
3/12	Injected 96-126 C.U. (60 mgm.)	Androstendion at 9:50 a.m.					
3/12					26.8	12.4	14.4
3/13	Injected 96-126 C.U. (60 mgm.)	Androstendion at 9:45 a.m.					
3/13	7.38	5.97	0.238	1.41	28.2	10.5	17.5
3/14	Injected 96-126 C.U. (60 mgm.)	Androstendion at 9:45 a.m.					
3/14					25.0	11.6	13.4
3/15	6.44	5.03	0.242	1.17	26.1	10.3	15.8
3/16					25.4	11.3	14.1
3/17	6.32	4.83	0.238	1.25	26.4	12.0	14.4
3/18					24.4	11.6	12.8
3/19	6.92	5.45	0.240	1.23	26.8	11.7	15.1
3/20							
3/21	7.08	5.64	0.248	1.19	26.9	12.5	14.4
3/22	7.29	5.66	0.240	1.29			

* End of urine period.

† Low value due to experimental error.

The dogs were thoroughly trained to their respective regimes. The surrounding temperature, which is recorded in the tables, was always

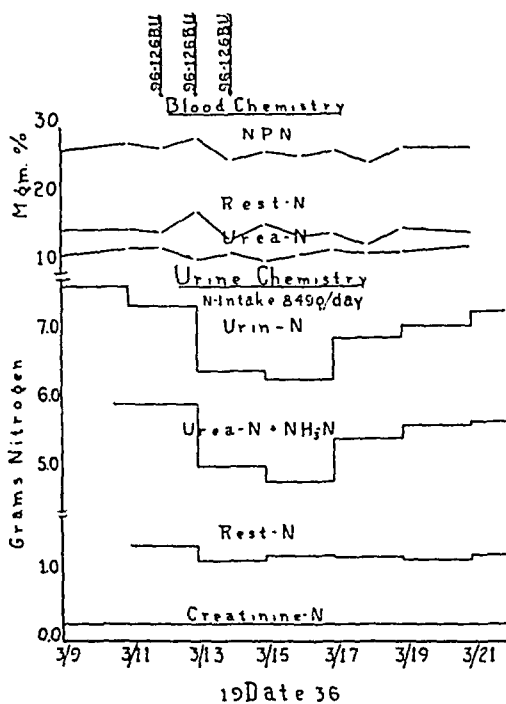


Fig. 1. Dog 2, experiment 2

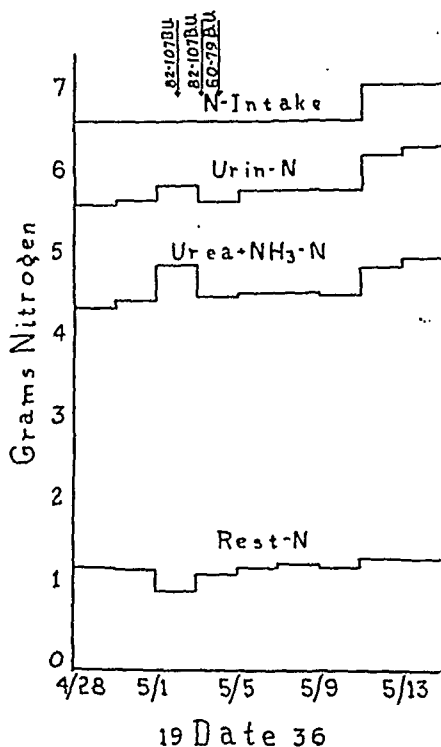


Fig. 2. Dog 6, experiment 1

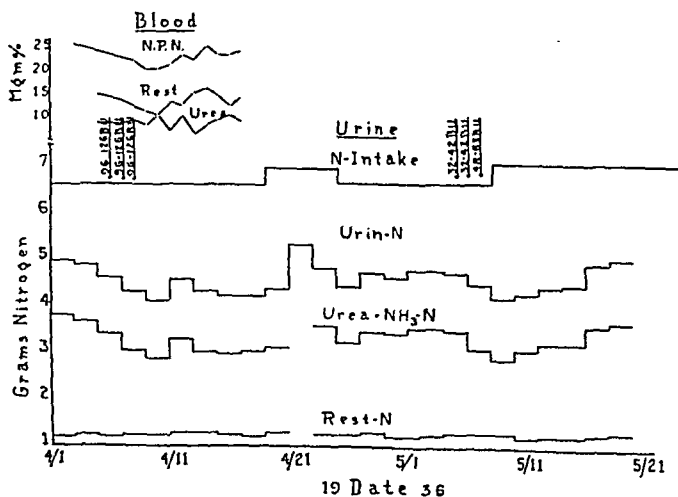


Fig. 3. Dog 1, experiments 1 and 2

within the normal range. The dogs were kept in the laboratory at all times.

The experiments on protein metabolism and energy metabolism were carried out simultaneously.

RESULTS. *Dog 2*: In experiment 1, table 2, only blood N.P.N. and blood urea were determined. The urinary nitrogen was not studied.

TABLE 4
Summary of nitrogen metabolism
Experiments 1 and 2, dog 1

DATE*	URINE CHEMISTRY—NITROGEN PER DAY				BLOOD CHEMISTRY		
	N-Intake	Total	Urea + NH ₃	Rest	N.P.N.	Urea N	Rest N
1936	grams	grams	grams	grams	mgm. per cent	mgm. per cent	mgm. per cent
4/3	6.63	5.01	3.82	1.19	26.2		
4/5		4.93	3.69	1.26	25.0	9.3	15.7
4/6	Injected 96-126 C.U. Androstendion at 11 a.m.						
4/7		4.66	3.46	1.20	23.6	9.3	14.3
4/7	Injected 96-126 C.U. Androstendion at 11 a.m.						
4/8	Injected 96-126 C.U. Androstendion at 11:10 a.m.						
4/9		4.33	3.07	1.26	20.7	9.0	11.7
					21.0	11.2	10.8
4/11		4.15	2.91	1.24	22.0	7.7	14.3
					24.1	10.9	13.2
4/13		4.61	3.32	1.29	23.1	7.2	15.9
					25.9	8.8	17.1
4/15		4.34	3.06	1.28	24.0	9.9	15.9
					23.9	11.0	12.9
4/17		4.24	2.99	1.25	24.8	9.7	15.1
4/19		4.25	3.05	1.20			
4/21		4.40	3.12	1.28			
4/23	6.98	5.33					
4/25		4.86	3.61	1.25			
4/27		4.46	3.24	1.22			
4/29	6.67	4.76	3.47	1.29			
5/1†		4.63	3.43	1.20			
5/4		4.81	3.54	1.27			
5/5	Injected 32-42 C.U. Androstendion at 11 a.m.						
5/6		4.72	3.42	1.30			
5/6	Injected 32-42 C.U. Androstendion at 11:05 a.m.						
5/7	Injected 48-63 C.U. Androstendion at 11:15 a.m.						
5/8		4.48	3.18	1.30			
5/10		4.19	2.90	1.29			
5/12	7.09	4.29	3.09	1.20			
5/14		4.46	3.23	1.23			
5/16		4.48	3.24	1.24			
5/18		4.95	3.63	1.32			
5/20		5.04	3.70	1.34			

* Date at end of period.

† Three-day period.

In experiment 2, table 3, total nitrogen, urea plus ammonia nitrogen, creatinine nitrogen and by difference rest nitrogen of urine are each re-

corded. Blood N.P.N. urea nitrogen and by difference rest nitrogen are also recorded.

The urinary nitrogen drops after injections reaching a minimum sometime between the 1st and 3rd day after the last injection and then begins to return to normal. The urea plus ammonia nitrogen parallels, except in the 1st period, the drop in total urinary nitrogen, the creatinine remains absolutely constant and the rest nitrogen also remains constant. The first period shows a high rest nitrogen and a corresponding low urea and NH_3 nitrogen which is undoubtedly experimental error. The blood constituents remain fairly constant in both experiments. It can be said, therefore, that the drop in urinary nitrogen is not due to a retention of non-protein nitrogen constituents.

TABLE 5
Summary of daily maximum nitrogen retentions

DOG	HORMONE	WEIGHT	C.U. INJECTED PER DAY	AVERAGE DAILY MAXIMUM N RETENTION	MAXIMUM N RETAINED PER KGM. PER DAY
		<i>kgm.</i>		<i>gram</i>	<i>gram</i>
1	Urinary	13	13-17*	0.6	0.05
	Urinary	13	25-34*	0.7	0.06
	Urinary	12	38-51*	0.6	0.05
	Urinary	12	25-34*	0.7	0.06
	Androstendion	15	96-126†	0.8	0.05
	Androstendion	15	32-42§	0.9	0.06
2	Urinary	22	25-34*	1.3	0.06
	Urinary	22	38-51*	1.3	0.06
	Urinary	22	13-17*	0.4	0.02
	Urinary	22	208-310†	1.3	0.06
	Androstendion	26	96-126†	1.4	0.05

* Injected daily over a period of 15-25 days. Maximum values obtained after first 5-day period.

† This amount administered for 3 successive days.

‡ Single injection.

§ This amount administered for 2 successive days and on third day 48-63 C.U injected.

The graphical presentation of the urinary and blood constituents during experiment 2 are presented in fig. 1.

Dog 1: The results for this dog, table 4 and fig. 3, show the same general effect as for dog 2, a decrease in urinary nitrogen, which is accounted for by the urea phase, the rest nitrogen remaining constant. The blood N.P.N. shows a slight drop subsequent to injection.

In the first experiment, table 4, on this dog, the hormone was administered in identical amount and manner as in experiment 2 (table 3) of dog 2.

In the second experiment, table 4, which was made directly after the first, the hormone was administered in an identical manner as in the first experiment, but in smaller amounts. On the first and second day, 32 to 42 C.U. or one-third the previous amount, and on the third day 48 to 63 C.U. or one-half the previous amount were administered. The maximum nitrogen retained per day, however, in both cases, is about the same—0.8 gram for experiment 1 and 0.9 gram for experiment 2. There is, nevertheless, a noteworthy difference. In experiment 2, as in experiment 1, dog 2, there is an immediate gradual return to basal after the point of maximum nitrogen retention is attained, while in experiment 1 there is a prolonged period of about 8 days of maximum nitrogen retention.

TABLE 6
Summary of nitrogen metabolism
Experiment 1, dog 6

DATE AT END OF PERIOD	N-INTAKE	URIN. N	$\frac{N}{\text{UREA} + \text{NH}_3}$	REST N
1936	grams per day	grams per day	grams per day	grams per day
4/30	6.67	5.66	4.40	1.26
5/1		5.72	4.50	1.22
5/2	Injected 82-107 C.U. Androstendion at 9:15 a.m.			
5/3	Injected 82-107 C.U. Androstendion at 9:15 a.m.			
5/3		5.90	4.94	0.96
5/4	Injected 60-79 C.U. Androstendion at 10:30 a.m.			
5/5		5.68	4.53	1.15
5/7		5.85	4.58	1.27
5/9		5.86	4.57	1.31
5/11		5.82	4.55	1.27
5/13	7.09	6.26	4.89	1.37
5/15		6.33	4.98	1.35

A comparison of the daily maximum nitrogen retention by dogs 1 and 2, table 5, shows that the amount retained by dog 2 is much greater than that by dog 1, even when identical amounts of hormone are administered. If, however, these values are converted to a weight basis, this difference disappears. The amount retained is the same for both dogs per unit of body weight, 0.05 to 0.06 gram nitrogen per kilogram. This is supported by a similar analysis of the results obtained with the urinary extracts in the previous study (1). Dog 2, in this study, showed an average daily maximum nitrogen retention of 1.3 gram or 0.06 gram N per kgm. per day, whether receiving 25 to 34 C.U. per day or 38 to 51 C.U. per day for more than 5 days or a single injection of 208 to 310 C.U. With a dosage of 13 to 17 C.U. per day for over 5 days, this same dog showed a daily nitrogen retention of only 0.3 to 0.4 gram or 0.01 to 0.02 gram N per kgm. per day,

which is only about one-fourth of that retained as the result of a dose twice as great. The results for dog 1 showed a maximum daily nitrogen retention of 0.6 to 0.7 gram or 0.05 to 0.06 gram per kgm. per day, with the administration of 13 to 17 C.U., 25 to 34 C.U., or 38 to 51 C.U. per day for periods of 15 to 25 days. It is noteworthy that dog 1 showed no significant decrease in the amount retained with the smallest dose, 13 to 17 C.U. per day, in contrast to dog 2. This is probably due to a smaller requirement of hormone by dog 1, which is much smaller than dog 2. There is, therefore, not only a similar weight relationship of the maximum daily nitrogen retention for both dogs, but also a similar quantitative effect is produced with the two different types of hormones. Furthermore, it might be stated, with due allowance for the fact that the data to demonstrate it are meagre, that dog 1 reaches this maximum state with one-half the dose required by dog 2. Since dog 1 is also one-half as heavy as dog 2, it would follow that the daily maximum nitrogen retention is proportional to the C.U. per kgm. This should be substantiated by more evidence.

Dog 6: The results obtained in the case of this normal dog are in marked contrast to the consistent results obtained with the castrate dogs. This dog received three successive daily injections of hormone in doses slightly less than the larger dose but much greater than the smaller administered to the castrate dogs. There is no indication of any effect on the nitrogen excretion. In fact there is an apparent rise of about 0.1 to 0.2 gram N per day, but this quite obviously is not significant. Also there is an apparent increase of urea N with a consequent decrease of rest N in the first 2 days of injection, but this change is only a little greater than daily variation and therefore cannot be ascribed to the injections, for there is no further change in the same direction due to a third injection. In fact, the urea and $\text{NH}_3\text{-N}$ and rest N are in the normal range again. These changes, therefore, must be attributed to experimental or metabolic variations. The apparent large increase of nitrogen excretion in the last two periods is due to an increased nitrogen intake caused by the use of a new batch of beef heart which had a higher percentage of nitrogen. These results are presented in table 6 and Fig. 2.

It must be said, therefore, that the administration of androstendion does not affect the protein metabolism of a normal dog.

Energy metabolism. Prior to carrying out the hormone experiments, a series of basal runs over a period of time was obtained to reaccustom the dogs to their respective regimes of metabolic determinations. Also just before each daily injection one or two metabolism periods were obtained followed by several more after the injection.

Although the protein metabolism was determined during all these experiments except experiment 1 on dog 2, no attempt is made to distribute the calories to the various constituents of fat, carbohydrate and protein.

We are primarily interested in noting whether there is any effect on the total energy metabolism.

Calories per square meter per hour are calculated according to the method of Cowgill and Drabkin (14).

Dog 1, whose metabolism was determined by means of a chamber at-

TABLE 7
Summary energy metabolism. Dog 1

DATE	NUMBER OF DETERMINATIONS	DURATION OF DETERMINATIONS	OBSERVED R.Q.	CALORIES PER HOUR	CALORIES PER SQUARE METER PER HOUR	AVERAGE TEMPERATURE OF CHAMBER	WEIGHT
1936						°C.	kgm.
3/31	2	10:29-12:18	0.77±0.01	18.7±1.0	30.2±1.6	23.5±0.1	15.2
4/1	2	10:03-12:08	0.76±0.01	18.8±1.3	30.3±2.0	22.5±0.1	
4/2	5	10:04- 2:58	0.77±0.01	18.7±0.7	30.1±1.1	22.0±0.5	
4/4	4	9:06- 1:05	0.79±0.01	18.1±1.2	29.2±2.0	22.7±0.2	15.0
4/6	2	9:00-10:53	0.77±0.04	18.6±1.2	30.0±2.0	21.2±0.1	
		Injected subcutaneously 96-126 C.U. (60 mgm.) Androstendion, 11:00					
	4	11:57- 4:40	0.77±0.03	20.0±0.6	32.2±1.0	22.4±0.4	
4/7	2	8:54-11:01	0.75±0.03	18.9±0.5	30.4±0.9	22.1±0.1	15.1
		Injected subcutaneously 96-126 C.U. (60 mgm.) Androstendion, 11 a.m.					
	4	12:22- 4:56	0.78±0.02	19.6±1.0	31.6±1.6	23.3±0.3	
4/8	1	9:04-10:03	0.73	18.6	30.0	23.2	16.0
		Injected subcutaneously 96-126 C.U. (60 mgm.) Androstendion, 11:10					
	4	12:31- 4:48	0.77±0.02	19.2±0.5	30.9±0.8	23.9±0.3	15.0
							15.4
4/9-4/27	13	9:00-12:00	0.76±0.02	18.5±0.4	29.8±0.7	23.5±0.5	15.5
5/1	2	9:27-11:21	0.74±0.01	18.5±0.4	29.8±0.7	24.5±0.1	15.4
5/4	2	9:38-11:42	0.75±0.01	18.7±0.5	30.1±0.8	23.6±0.1	
5/5	2	8:50-10:51	0.77±0.01	18.0±1.0	29.0±1.6	24.1±0.1	
		Injected 32-42 B.U. (20 mgm.) Androstendion, 11 a.m.					
	4	11:45- 4:17	0.80±0.01	18.0±0.8	29.0±1.2	24.4±0.2	
5/6	2	8:54-10:57	0.75±0.01	18.4±0.2	29.7±0.3	24.1±0.1	
		Injected subcutaneously 32-42 C.U. (20 mgm.) Androstendion 11:05 a.m.					
	4	12:07- 4:19	0.79±0.02	17.9±0.5	28.8±0.8	24.1±0.1	
		Injected subcutaneously 48-63 (30 mgm.) Androstendion, 11:15					
5/7	3	12:27- 3:53	0.75±0.03	18.6±0.9	30.0±1.5	24.5±0.5	

tached to a Benedict Universal apparatus, was injected with the prescribed dose after two successive periods of approximately 1 hour each were obtained, returned to his cage for a short rest period of approximately $\frac{1}{2}$ hour, placed again in the metabolism chamber and after a preliminary period of approximately $\frac{3}{4}$ hour, 4 successive determinations of approximately one hour each were made.

As can be seen from table 7, dog 1 shows no inclination to increase or decrease its energy metabolism, either immediately after injection or 24 hours after injection, nor is there any later effect due to the repeated

TABLE 8

Summary energy metabolism. Dog 2

Weight 26.0 ± 0.1 kgm. Length 90 cm. Surface area 0.83 sq. m.

DATE	TIME BEGINNING OF PERIOD	ROOM TEMPERATURE	OBSERVED R.Q.	CALORIES		VENTILATION RATE	RECTAL TEMPERATURE	RESPIRATION
				Per hour	Per sq. m. per hour			
1936		°C.				liters per hour	°C.	
2/14-2/15	9:25- 2:14	24.1 ± 0.4	0.73 ± 1	25.3 ± 0.3	30.4 ± 0.4	170.2 ± 9.4	39.0	14 ± 2
2/17	10:01	23.4	0.73	25.5	30.7	175.6	39.1	14
	10:19	Injected subcutaneously 32-42 C.U. Androstendion						
	1:00	23.7	0.71	25.9	31.2	180.1	39.0	14
	3:38	23.6	0.74	25.6	30.8	199.1	39.1	16
2/18	9:06	23.4	0.75	25.1	30.2	180.9	39.0	14
	9:24	Injected subcutaneously 32-42 C.U. Androstendion						
	10:31	23.8	0.74	25.5	30.7	178.6		14
	3:48	24.4	0.73	26.3	31.6	170.4	39.0	10
2/19	9:23	25.1	0.75	25.7	30.9	213.6	39.0	17
	10:15	Injected subcutaneously 32-42 C.U. Androstendion						
	11:41	24.3	0.72	24.1	29.0	163.0		14
	2:24	24.4	0.72	25.8	31.0	173.2	39.0	11
	4:55	24.8	0.75	25.9	31.2	197.3		14
2/20	9:50	25.2	0.74	25.3	30.4	178.2	39.0	12
2/21-2/24*	10:05-10:31	24.7 ± 0.5	0.74 ± 0.1	24.3 ± 0.3	29.3 ± 0.4	174.7 ± 5.1	38.9	13 ± 1
3/ 7-3/11†	8:28- 4:17	25.7 ± 0.7	0.74 ± 0.2	23.5 ± 1.0	28.3 ± 1.2	162.5 ± 12.6		12 ± 1
3/12	8:55	25.2	0.74	23.1	27.8	161.6		13
	9:50	Injected subcutaneously 96-126 C.U. Androstendion						
3/13‡	8:51- 9:10	24.5 ± 0.0	0.73	23.7 ± 0.1	28.5 ± 1.2	142.0	38.3	10
	9:45	Injected 96-126 C.U. subcutaneously Androstendion						
	11:31	25.2	0.77	24.5	29.5	186.5	38.2	14
	2:07	25.8	0.74	22.2	26.7	138.4		11
	3:34	25.8	0.75	23.0	27.7	164.1		13
3/14	9:17	24.3	0.75	22.7	27.3	169.3	38.0	13
	9:45	Injected subcutaneously 96-126 C.U. Androstendion						
	1:09	25.1	0.78	23.0	27.7	165.6	38.1	13
	4:38	25.7	0.74	25.3	30.4	191.0		16
3/15-3/21§	9:36-10:50	24.4 ± 0.4	0.74 ± 0.01	23.4 ± 0.9	28.1 ± 1.1	161.1 ± 5.7	38.0 ± 0.1	13 ± 1

* Average of 3 determinations.

† Average of 9 determinations.

‡ Average of 2 determinations.

§ Average of 4 determinations.

injections. The dog's weight shows a slight increase which probably is not significant.

The conditions during the entire experimental period were well con-

trolled. The metabolic determinations were made in as nearly equal periods of time as possible, the calorimeter chamber temperature was kept within favorable limits, in fact almost constant at $23 \pm 1^\circ\text{C}$.

Dog 2: After a preliminary period of basals which indicated that the dog had become accustomed to his new helmet, the experiments were started. Prior to each injection a metabolic determination was obtained and after the injection 2 or 3 more determinations were obtained with the exception of the experiment on March 2, 1936. Subsequent determinations were not obtained on this day because the dog proved refractory at several attempts. The results, table 8, show no tendency for an increase in metabolism at any time during the entire experimental period. In fact, there is a slight drop of about 8 per cent between the first series of experiments, February 14 to February 24, and the second series, March 7 to March 21, which is accompanied by a similar drop in rectal temperature. This is difficult to attribute to the effects of the injections, but seems rather to be a result of a better adaptation of the dog to his new helmet as is evidenced by a lower rectal temperature.

DISCUSSION. The results in this study are in complete confirmation of the results obtained in the previous study (1). It cannot be said, therefore, that there are any qualitative or quantitative differences between the urinary extracts of male hormone and the synthetic male hormone, androstendion, in their relation to the energy and protein metabolism of castrate dogs, although they are chemically different.

The failure to increase the maximum nitrogen retention with the administration of increased amounts of male hormone indicates that any male hormone administered beyond a certain dosage is unnecessary and wasteful. Apparently some controlling mechanism renders the excess male hormone unarmful. Fear of untoward effects from excessive doses of male hormone should therefore not obstruct the clinical application of this material.

The similar quantitative maximum daily nitrogen retention, obtained by a comparison of the results of this study on androstendion with the results of the previous study on the urinary hormone, was not anticipated. It was rather expected that the difference in the chemical properties of the materials used in the two studies would show a similar qualitative effect but a different quantitative effect. Ruzicka, Goldberg and Rosenberg (15) (see also Ruzicka and Wettstein, 6) in an analysis of the relationship of chemical structure of the various natural and synthetic male hormones, found that substances containing a keto group in the "three" position, as in androstendion, have a greater effect on the physiological reaction of the accessory sex organs of castrate rats, than do the substances containing a hydroxyl group in this position, as in the urinary product.

The results of the study of the blood N.P.N. which never showed an increase, and in fact showed a decrease of about 15 per cent (dog 1, experiment 1), indicate that the decrease in urinary nitrogen is not due to a retention of non-protein nitrogen constituents. It rather indicates that there is a utilization of circulating amino acids for rebuilding new tissue.

The interesting results obtained on the protein metabolism of the normal dog are worthy of remark. This dog with its testes intact is presumably producing enough male hormone to maintain its accessory sex organs in a normal physiological state. Not so the castrate dogs. When male hormone is administered to these dogs, a stimulus is provided for the restoration of the accessory sex organs which is met by a mobilization of the circulating amino acids to regenerate the atrophied organs. Such retention of protein beyond regeneration would seem to be confined to structures atrophied by castration for maintenance of protoplasm and production of their secretions. It appears also, that if the physiological requirements for male hormone are met, any excess male hormone will not be effective in producing further changes in protein metabolism. This hypothesis is further supported by the fact that in the previous study it was noted that the nitrogen retained reached a maximum within 5 days and that a further increase of the daily dose or the extension of the injections did not produce an increased nitrogen retention, but only maintained this maximum. This phenomenon is similar to that observed in the present study.

The male hormone illustrates well the action of a specific growth regulator. When present in adequate amount it induces anabolism of protein (nitrogen retention) in specific structures. Inherent limitations of growth, tracing back to the germinal chromosomes, however, prevent continuous uncontrolled expansion of these structures in proportion to the amount of hormone present. In all probability the full development of the several accessory sex glands under the influence of the animal's own normally produced hormone cannot be exceeded after castration under the effects of synthetic or extracted hormone. Excess growth beyond the normal limits would be teratological.

Relationship in regulatory effects between sex hormones and the growth hormone of the pituitary naturally are suggested, but this is a problem of itself. As a first step in the elucidation of this problem, experiments are now underway to find a maximum dosage, if any, beyond which there will be no further increase in the size of the accessory glands of castrate rats. Would the gonadotropic hormone from the anterior pituitary be able to condition the accessory structures for a larger effect? Many other questions can be raised for which there is yet no answer.

The energy metabolism in both castrate dogs remained constant within a maximum range of 10 per cent. The decreased caloric output due to

decreased protein metabolism was very probably compensated for by an increased fat or carbohydrate metabolism or of both. The decreased energy from protein, however, is not very great, a maximum of about 1.5 calories per hour for dog 2, for 1 or 2 days, and at all other times for both dogs it is much less. This decrease falls well within the range of experimental error.

The confirmation of the results obtained on energy metabolism with urinary extracts plus unpublished similar data on human subjects, leads only to one conclusion, namely, the endocrine function of the testes as represented by the male hormones cannot be considered as a rejuvenating mechanism in the calorigenic sense. In order to make this more conclusive, a similar study with testosterone will be undertaken.

SUMMARY

The results of injections of androstendion in castrate adult male dogs confirm the previous findings with the use of urinary male hormone extracts.

The protein metabolism decreases as a result of injection. The nitrogen retention, which is borne by the urea fraction of the urine, is attributed for the present to the regeneration of the accessory sex organs and possible production of their secretions.

The daily maximum nitrogen retained and the amount of male hormone necessary to produce this effect are proportional to the body weight and are the same for the urinary product and androstendion, although the two are chemically different.

The blood N.P.N. and urea show if anything a decrease, but never an increase.

The protein metabolism of a normal dog in contrast to the castrate dogs shows no effects.

It is suggested that there is a maximum physiological requirement of male hormone, beyond which additional hormone has no effect.

The energy metabolism of the castrate dogs is not affected. Thus further evidence is provided that the male hormones cannot be considered as calorigenic agents.

REFERENCES

- (1) KOCHAKIAN, C. D. AND J. R. MURLIN. *J. Nutrition* **10**: 437, 1935.
- (2) BUTENANDT, A. AND H. KUDSZUZ. *Ztschr. physiol. Chem.* **237**: 75, 1935.
- (3) RUZICKA, L. AND A. WETTSTEIN. *Helv. Chim. Acta.* **18**: 986, 1935.
- (4) BUTENANDT, A. AND H. DANNENBAUM. *Ztschr. physiol. Chem.* **229**: 192, 1934.
- (5) BUTENANDT, A. AND G. HANISCH. *Ztschr. physiol. Chem.* **237**: 89, 1935.
- (6) RUZICKA, L. *Helv. Chim. Acta.* **18**: 1264, 1935.
- (7) STRAIN, W. H. AND W. M. ALLEN. *Ind. Eng. Chem. Anal. Ed.* **17**: 443, 1935.

- (8) VAN SLYKE, D. D. AND G. E. CULLEN. J. Biol. Chem. 24: 117, 1916.
- (9) FOLIN, O. J. Biol. Chem. 17: 469, 1914.
- (10) HAWK, P. B. AND O. BERGEIM. Practical physiological chemistry. Blakiston's Sons & Co., Philadelphia, 9th ed., 368, 1926.
- (11) LIEBHOF, S. L. AND B. S. KAHN. J. Biol. Chem. 83: 347, 1929.
- (12) NASSET, E. S. This Journal 101: 194, 1932.
- (13) BENEDICT, F. G. Abderhalden Handbuch der Arbeitsmethoden 4: 465, 1933.
- (14) COWGILL, G. R. AND D. L. DRABKIN. This Journal 81: 36, 1927.
- (15) RUZICKA, L., M. W. GOLDBERG AND H. R. ROSENBERG. Helv. Chim. Acta. 18: 1487, 1935.

UREA CLEARANCE AND PROTEINURIA DURING EXERCISE

ARTHUR B. LIGHT AND CLARK R. WARREN

From the Medical Department of The Lawrenceville School, Lawrenceville, New Jersey

Received for publication July 22, 1936

The presence of increased amounts of protein, red cells and casts in the urine secreted during exercise has been frequently demonstrated. These findings, however, are not regarded with any serious apprehension when the kidney is known to function quite satisfactorily during normal conditions. The presence of albuminuria during exercise was probably first discovered by Leube (1) in 1878, although credit is given to De la Camp by Schmidt and Kohlrausch (2). A decrease in kidney function as measured by the urea clearance test has also been found during increased muscular activity, by Addis and Drury (3), Mackay (4), Van Slyke, Alving and Rose (5) and Benzinger (6). The purpose of the studies reported in this paper was to determine if possible any correlation between the degree of albuminuria and changes which might occur in the urea clearance during vigorous exercise.

Our subjects were all healthy young males attending the Lawrenceville School, ranging in age from 14 to 20 years. They lived under quite identical conditions, including diet. Three sports, football, soccer and basketball were chosen as the form of exercise, primarily because these games are popular with the normal boy; secondarily because regulation games are long enough to secure adequate specimens of urine for analysis and finally to ascertain any manifest difference between these three sports. All games were played according to interscholastic rules, supervised by competent officials. The existing rivalry was so intense as to force each boy to play to the limit of his endurance.

EXPERIMENTAL. At least one and frequently two boys from each team, playing in the most strenuous positions, were selected as subjects. Samples of blood and urine were discarded unless the subject played the full regulation game, and voided immediately after the cessation of exercise. Despite the fact that the sample of blood was collected and the urine discarded at the last possible moment before the game began, and another sample of blood obtained and the urine collection made immediately after the game in the gymnasium, actual exercise represents only about one-half of the collection time. The balance of the period was consumed by rest periods between quarters and halves and "times out."

Normal clearances for each subject were obtained in the morning in the routine procedure recommended by Möller, McIntosh and Van Slyke (7). Their breakfasts consisted of fruit, cereal and milk. They spent the interim between collections either in their rooms or attending classes. The two collection periods varied from three-quarters to one hour each.

The aeration method, using Squibb's urease was used to determine the blood and urine urea nitrogens. The protein in the urine was determined by Folin's (8) quantitative method. All samples were analyzed in duplicate.

RESULTS. Complete studies were carried out on twenty-nine boys, eleven subjects playing football, six soccer, and twelve basketball. McIntosh, Möller and Van Slyke's (9) line chart was used to correct for the age and height of the subjects. All clearance values were calculated as per cent of normal. Minute volumes of urine below 2 cc. per minute were computed as standard clearances, volumes above this figure as maximum.

The average normal clearance for the entire group of subjects was 136.4 per cent with a standard deviation of 43 and a standard error of the mean at ± 8 . With the normal value placed at 100 by Van Slyke, this age group appears to have higher clearance values than those for adults and the lower age group reported by Cullen, Nelson and Holmes (10). The lowest normal clearance value obtained was 85 per cent, the highest 241 per cent. The blood urea nitrogen averaged 11.4 mgm. per 100 cc. of blood, ranging from a minimum of 6.1 mgm. to a maximum of 15.4 mgm.

During exercise, the group playing football cleared 47.5 per cent of their normal, soccer 53.3 per cent and basketball 37.3 per cent. Despite the greater percentage decrease in the clearance found in the group playing basketball as compared to the groups playing soccer and football, the results when analyzed statistically show the standard errors of the various means of the three groups to be such that would indicate that there is no significant difference between them. The average blood urea nitrogen increased from 8.9 mgm. to 10.6 mgm. per 100 cc. during football, 12.1 mgm. to 12.5 mgm. during soccer and 12.8 mgm. to 13.3 mgm. during basketball.

The amounts of albumen excreted by the different subjects in the various sports, computed on the basis of half-hour periods, varied tremendously. The type of sport did not appear to make any essential difference. No correlation appeared to exist between the percentage drop from the individual's normal clearance and the degree of proteinuria. A careful study of the posture, normal blood pressure, deviation from the normal weight for age and height for each subject, failed to offer any solution for the widely different amounts of albumen excreted and computed for half-hour periods.

The average urine minute volumes during the periods of exercise re-

mained remarkably constant, being 0.43 cc. per minute for football, 0.51 cc. for soccer, and 0.46 cc. for basketball. Attempts to link the degree of proteinuria with the minute volumes failed completely.

DISCUSSION. The definite decrease of the urea clearance in every one of our subjects is in accord with the findings of the previously mentioned investigators (3) (4) (5) (6). The failure to show any correlation between changes in urea clearance and proteinuria induced by vigorous exercise are interesting inasmuch as Medes and Berglund (11) were unable to correlate the proteinuria ensuing when subjects assumed the lordotic position with the concomitant changes in creatinine clearance.

The inconstancy of the proteinuria resulting from vigorous exercise has already been noted by Roberts (12). Our results not only corroborate his findings, but a series of unpublished estimations made from a group of subjects during each game of an entire season bears out this inconstancy. Neither the excitement before the game, the closeness of the match nor the minute volume of urine secretion seemed to play any part in the amount of protein excreted. In general, however, there was a gradual decrease in the amounts excreted as the seasons progressed, suggesting training and physical fitness as a possible contributing factor.

Hellebrandt, Walters and Miller (13) were unable to raise the urine minute volume during vigorous exercise, by increasing the fluid intake. We attempted this same procedure in a number of our subjects. Quite frequently the subjects flatly refused to drink more than their accustomed amounts. In a few instances, we were able to raise the fluid intake considerably. The minute volumes of this latter group, however, remained consistently low, and practically the same as those obtained from the rest of the group.

SUMMARY

1. A definite fall in urea clearance was noted in every one of our subjects engaged in playing regulation football, soccer and basketball games.

2. No correlation existed between the degree of proteinuria induced by exercise and the changes in the urea clearance values obtained during this same period.

3. With few exceptions, the urine minute volume is quite low and uniform for the three different sports.

4. There is no significant difference in the fall of the urea clearances obtained between the three sports.

5. The average normal clearances obtained from this age group (males 15-20 years) 136.4 per cent is definitely higher than that for adults or lower ages than our group.

REFERENCES

- (1) LEUBE, W. Virchow's Arch. f. path Anat. 72: 145, 1878.
- (2) SCHMIDT, F. A. AND W. KOHLRAUSCH. Physiology of muscular exercise. F. A. Davis, Philadelphia, 1931.
- (3) ADDIS, T. AND D. R. DRURY. J. Biol. Chem. 55: 629, 1923.
- (4) MACKAY, E. M. J. Clin. Invest. 6: 505, 1928.
- (5) VAN SLYKE, D. D., A. ALVING AND W. C. ROSE. J. Clin. Invest. 11: 1053, 1932.
- (6) BENZINGER, T. Arbeitsphysiologie 8: 142, 1934.
- (7) MOLLER, E., J. F. MCINTOSH AND D. D. VAN SLYKE. J. Clin. Invest. 6: 427, 1928.
- (8) FOLIN, O. Laboratory manual of biological chemistry. New York, Appleton, 1925.
- (9) MCINTOSH, J. F., E. MÖLLER AND D. D. VAN SLYKE. J. Clin. Invest. 6: 467, 1928.
- (10) CULLEN, G. E., W. E. NELSON AND F. E. HOLMES. J. Clin. Invest. 14: 563, 1935.
- (11) MEDES, G. AND H. BERGLUND. The kidney in health and disease. p. 462. Lea & Febiger, Philadelphia, 1935.
- (12) ROBERTS, A. M. J. Clin. Invest. 14: 31, 1935.
- (13) HELLEBRANDT, F. A., C. E. WALTERS AND M. L. MILLER. This Journal 116: 168, 1936.

THE CONCENTRATION OF NUCLEATED CELLS IN THE BONE MARROW OF THE ALBINO RAT¹

GEORGE E. FARRAR, JR.²

From the Division of Pharmacology, Food and Drug Administration, Washington, D. C.

Received for publication July 25, 1936

In the study of the chronic toxicity of small amounts of materials such as lead and arsenic, a more delicate measure of the status of the bone marrow is needed. The detection of slight changes in histological sections of bone marrow is extremely difficult. A method of counting the total number of cells per cubic millimeter volume of marrow is essential for the detection of minimal toxic effects. Several methods (1), (2), (3), (4) are available for the differential classification and enumeration of the different types of cells present in the marrow. Of these methods the first (1) has proved the most satisfactory in this study. In this method cover slip films, prepared from a suspension of bone marrow in fresh blood serum of the same species of animal, are dried in air and stained with Wright's blood stain. The present paper is a report of the observations made on a series of normal animals with respect to the number of nucleated cells in the marrow at various age periods.

The animals used in this study were albino rats, stock animals of both sexes, and varied in age from 4 days to 13 months. All were of the Osborne-Mendel stock, although part had been raised in the Division of Pharmacology and part in Vitamin Division, where different stock diets were in use. No animals were included that were on inadequate diets or that had received any toxic substance. For counting, a modification of the method employed by Isaacs (1) has been used on the marrow of the femur. Because of the absence of bone spicules, it is a relatively simple matter to remove the marrow, either for dilution in a blood-counting pipette or for fixation and section without decalcification. The details of this method, which has been developed and used as a rapid and routine autopsy procedure for the enumeration of the total number of nucleated cells per cubic millimeter of marrow, are presented in the next paragraph. The number of mature red cells has not been determined.

¹ This study is a part of a comprehensive investigation of the toxicity of small amounts of lead and arsenic which is being carried out by the Division of Pharmacology of the Food and Drug Administration under the direction of Dr. Erwin E. Nelson and Dr. Herbert O. Calvery.

² Associate Pharmacologist.

EXPERIMENTAL. The procedure which has been found satisfactory is as follows: the shaft of the femur is dissected free of muscle and the distal end of the bone cut off, exposing the marrow in the shaft. With the usual red-blood-cell-diluting pipette, marrow is aspirated up to the first 0.001 mark, and then diluted to the 1.01 mark with 1 per cent acetic acid. This diluted material is then shaken in a mechanical shaker for 30 minutes. The counting chamber used for blood cell counts is filled, a few minutes allowed for settling, and the cells in three of the 1-square-millimeter areas counted. With the average of these three squares, the thickness and dilution factor is 10,000.

With this procedure the values recorded in the chart were obtained

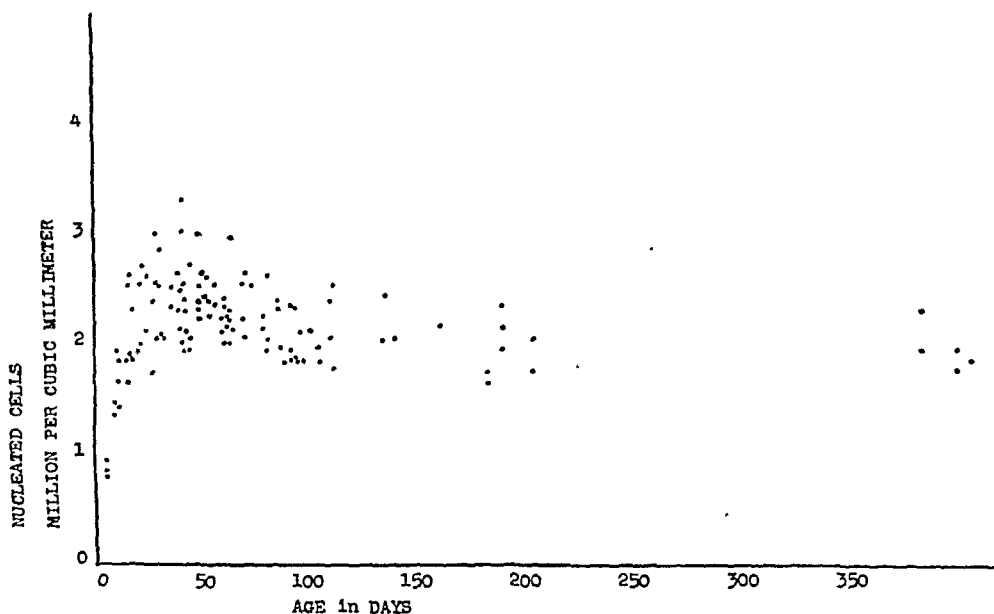


Fig. 1. The number of nucleated cells per cubic millimeter of the bone marrow of the femur of the white rat at different ages.

from 125 stock colony albino rats in which no abnormalities were found at autopsy. (See figure.) Up to about 3 weeks of age (23 animals), a progressive increase in the concentration of nucleated cells occurs. Between 3 weeks and 3 months, the average number per cubic millimeter of femoral marrow (70 rats) was 2.40 million with 84 per cent of the observations falling between 2.00 and 2.75. In observations on 32 rats from 3 months of age up to 13 months, the average count was 2.02 million. In this period there was qualitatively an increase in the amount of fat in the marrow.

DISCUSSION. The accuracy and validity of this technic were scrutinized from several points of view—namely, the uniformity of suspension in the

diluting pipette and the counting chamber, the adequacy of the size of the marrow sample employed, the agreement obtained in counts from both femurs and from different parts of the same femur. In many tests of these points the maximum single variation was 530,000 cells per cubic millimeter; most of the differences were of the order of 150,000 cells per cubic millimeter. In spite of the summation of errors that undoubtedly occurs, the agreement in counts is sometimes quite remarkable. For example, six males of the same litter, 13 weeks old, showed counts of 1.87, 1.84, 1.89, 1.87, 1.84, and 1.94 million nucleated cells per cubic millimeter, respectively; three males and three females from one litter just over 8 weeks of age showed 2.36, 2.01, 2.19, 2.41, 2.32, and 2.02 million nucleated cells, respectively; and in 2 males and 2 females from a litter 6 weeks old, 1.93, 2.12, 2.41, and 2.30 million, respectively. The last two of these groups illustrate the similarity in values obtained for both sexes of the same age and litter. Among 32 groups consisting of from 2 to 8 rats each, the average difference between litter mates of the same age was 281,000 cells per cubic millimeter. This approximates the accuracy obtained in counting the red blood cells in the peripheral blood.

The variations observed in certain pathological conditions are reserved pending the completion of more extensive studies. It may, however, be mentioned that in inanition of varying degrees there occurs a rapid and large decrease in the number of nucleated cells which is confirmed by histological examination of bone marrow sections.

SUMMARY

Examination of the bone marrow of the femur in the albino rat by a modification of the methods used for counting blood cells gives results which are reproducible within a range of 2 to 3 thousand nucleated cells per cubic millimeter in litter-mate rats of the same age. In normal rats there is an increase in the number of nucleated cells in the bone marrow during approximately the first 3 weeks of life, after which the count remains constant or falls slightly.

REFERENCES

- (1) ISAACS, R. Trans. Assoc. Am. Physicians 50: 249, 1935; also Am. J. Med. Sci. (in press).
- (2) SABIN, F. R. Physiol. Revs. 8: 191, 1928.
- (3) KRUMBHAAR, E. B. AND R. P. CUSTER. Am. J. Med. Sci. 189: 630, 1935.
- (4) STASNEY, J. AND G. M. HIGGINS. Anat. Rec. 63: 77, 1935.

THE BLOOD CLEARANCE AND RENAL EXCRETION OF BILE ACIDS FOLLOWING THE INTRAVENOUS INJECTION OF CHOLIC AND DESOXYCHOLIC ACIDS^{1,2}

S. S. LICHTMAN

*From the Division of Laboratories and from the Medical Service of Dr. George Bachr,
The Mount Sinai Hospital, New York*

Received for publication July 27, 1936

The metabolism of the bile acids has been studied by the feeding or intravenous injection of whole bile or of conjugated cholic acid. The injected bile acid was found to leave the blood and appear in the bile within two hours (1). A small fraction escaped in the urine (1, 2).

New methods for the estimation of all types of bile acids, especially desoxycholic acid, were required, in order to obtain further information on the metabolism of bile acids. The author devised a procedure, based in principle upon the hemolytic properties of the bile acids (3). This method estimates the aggregate hemolytic effects of the individual bile acids in the body fluids and expresses them in terms of desoxycholic acid, the most actively hemolytic of the tested bile acids. The probable presence of this bile acid in the blood (4) and the possible physiological and clinical significance of increases of this bile acid in the body fluids has already been suggested (3).

The blood clearance and renal elimination of bile acids following the injection of desoxycholic acid was compared with that of cholic acid to determine essential differences in physiological or metabolic behaviour between these principal types of bile acids occurring in man. Certain biological differences have already been noted (5).

EXPERIMENTAL. The sodium salts of desoxycholic acid (Riedel-de Haen) and cholic acid were injected rapidly into the saphenous or ear veins of normal fasting dogs. The doses used are stated in terms of the acids. Concentrations of desoxycholate as high as 20 per cent and of cholate as high as 16 per cent were used dissolved in physiological saline or in 0.1 molar phosphate buffer solution, pH 7.0. The desoxycholate solution in buffer required readjustment to pH 7.0. The results obtained were the same whether saline or buffer solutions were used.

¹ Aided by Grant no. 342 from the Committee on Scientific Research, American Medical Association.

² Mr. I. J. Madorsky rendered expert technical assistance.

Blood samples were collected by arterial puncture. Urine was collected quantitatively by catheter. Water was fed by tube, 90 minutes before injection to assure standard fluid intake and ample urinary output. The bladder was emptied immediately before injection. Mature dogs weighing 12 to 24 kilos were used. The doses were well tolerated aside from prompt hemoglobinuria following the injection of larger doses. Body weight was usually maintained days after the injection. In most instances fresh dogs were used for each experiment. Anesthesia was not required.

Samples of blood and urine were collected 20, 60, 120, 180, 240 minutes after injection. Nine cubic centimeters of urine and whole oxalated blood were examined in duplicate. Results were read by the artificial light of a 150 Watt daylight electric bulb transmitted through glazed glass, the test tubes being held directly against the glass surface. A special sectional test tube rack was devised for this purpose (6). The blood and urine of normal dogs were usually found to contain one milligram per cent or less, if any, of desoxycholate hemolytic equivalents. In several instances the bile acid content of the blood reached 2.0 mgm. per cent, that in the urine 3.5 mgm. per cent, figures approximating those in man (3).

Rate of elimination of bile acids from the blood. Doses representing 10, 20, 40, 60, 80, and 100 mgm. of desoxycholic acid were injected intravenously. The injection was completed within a minute. The findings in the blood at timed intervals are recorded in table 1. A definite elevation in the blood concentration was first noted when 20 mgm. of desoxycholic acid per kilo were injected. With larger doses, a maximum blood concentration of six milligrams per cent desoxycholate hemolytic equivalents was reached. The first determination, made 20 minutes after the injection, indicated an elevated concentration. Determinations made at 60 minutes after injection indicated a slight rise in bile acid content of the blood above the 20 minute level in some instances. At 120 minutes, the concentration fell but was still raised above the control level. The normal blood level was reached in 180 minutes. In some instances, the blood concentration again rose slightly at the end of 240 minutes after injection (expts. 3, 6, 12).

The blood is cleared in less than 20 minutes following the injection of restricted doses of cholic acid. When, however, as much as 300 mgm. of cholic acid per kilo was injected, the blood concentration reached six milligrams per cent of desoxycholate hemolytic equivalents. The blood rise continued for 180 minutes (expt. 11).

Urinary excretion following the injection of bile acids. The bile acid concentration of the urine at time intervals corresponding with those in the blood is recorded in table 1. Doses of 20 and 40 mgm. desoxycholic acid which caused definite rises in blood concentration caused practically no change in urinary concentration. The reverse is true following the injection of cholic acid. A markedly increased bile acid excretion reaching a

TABLE 1

Blood clearance and urinary excretion of bile acids following the intravenous injection of desoxycholic and cholic acids

EXPERIMENT NUMBER	BILE ACID	INTERVALS AFTER INJECTION											
		Control		20 minutes		60 minutes		120 minutes		180 minutes		240 minutes	
		Blood	Urine	Blood	Urine	Blood	Urine	Blood	Urine	Blood	Urine	Blood	Urine
		mgm. per kilo	mgm. per 100 cc.*	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.
	Desoxy- cholic acid												
1	10	0.0	3.5	0.5	q.n.s.	1.0	q.n.s.	0.5	1.0	0.5	1.0	0.0	2.5
2	20	0.0	3.5	2.5	2.0	3.5	0.0	2.5	1.0	1.0	1.0	2.0	3.0
3	40	0.0	0.5	5.0	2.0	6.0	2.0	3.0	1.0	1.5	1.0	3.5	0.0
4	60	0.0	0.0	3.5	3.0	5.5	2.5	2.0	0.0	0.0	0.0	1.0	0.0
5	80	2.0	2.0	3.5	20.0	3.5	10.0	2.5	0.5	2.5	q.n.s.	1.0	q.n.s.
6	100	0.0	0.6	3.5	3.5	6.0	2.0	2.0	1.0	0.0	0.0	2.5	0.0
	Cholic acid												
7	40	0.5	0.5	0.5	1.0	1.0	2.5	0.5	1.0				
8	60	0.5	0.5	2.0	8.0	2.0	2.5	0.5	0.0	0.5	0.5	0.5	1.0
9	100	0.5	0.0	0.5	5.5	0.5	4.0	0.5	2.0	1.5	0.0	1.0	1.0
10	170	1.0	0.0	0.5	12.0	0.0	7.0	0.0	0.5	1.0	0.5	0.0	1.0
11	300	0.0	0.0	6.0	71.0	5.0	71.0	5.0	75.0	5.0	49.0	2.0	25.0
	Desoxy- cholic												
12	40	0.5	0.0	3.5	13.0	6.0	0.0	3.5	0.5	0.5	0.0	2.5	5.0
	Cholic												
	170												

* Concentrations, milligrams per 100 cc., expressed as desoxycholate hemolytic equivalents. To translate into cholate hemolytic equivalents multiply by factor of 7.5.

Protocols. Experiment 1. Dog 1, bitch, 12.7 kilos. Four per cent solution of desoxycholate was used. The total amount injected was 130 mgm. The total urine voided in four hours was 42 cc.

Experiment 2. Dog 2, male setter, 18.6 kilos. Two per cent desoxycholate was injected. The total amount injected was 360 mgm. The total amount of urine voided in four hours was 190 cc.

Experiment 3. Dog 3, male collie, 18.1 kilos. A 4 per cent desoxycholate solution was injected. The total amount injected was 720 mgm. A total of 355 cc. of urine was voided in four hours.

Experiment 4. Dog 4, bitch, 17.1 kilos. A 10 per cent desoxycholate solution was injected. The total amount injected was 1020 mgm. The total voided was 127 cc.

Experiment 5. Dog 5, bitch, 12.7 kilos. A 10 per cent desoxycholate solution was

injected. The total amount injected was 1020 mgm. Fifteen cubic centimeters of urine were voided in four hours.

Experiment 6. Dog 6, bitch, 15.3 kilos. A 20 per cent desoxycholate solution was used. The total amount injected was 1530 mgm. A total of 45 cc. of urine was voided.

Experiment 7. Dog 7, female hound, 17.4 kilos. An 8 per cent cholate solution was used. A total of 700 mgm. was injected. A total of 270 cc. of urine was voided in two hours.

Experiment 8. Dog 8, bitch, 17.3 kilos. A 10 per cent cholic acid solution was injected. The total amount used was 1040 mgm. Two hundred seventy cubic centimeters of urine were voided in four hours.

Experiment 9. Dog 9, female hound, 16.5 kilos. A 16 per cent cholate solution was injected. The total amount injected was 1600 mgm. Three hundred forty-five cubic centimeters of urine were voided in four hours. The equivalent of 80 mgm. of cholic acid was excreted in the urine in 60 minutes after the injection.

Experiment 10. Dog 10, male police dog, 23.7 kilos. Sixteen per cent cholate solution was injected. The total amount injected was 4030 mgm. Five hundred and thirty cubic centimeters of urine were voided in four hours. The equivalent of 105 mgm. of cholic acid was excreted in the urine in 60 minutes after the injection.

Experiment 11. Dog 8, bitch, 15.6 kilos. Ten per cent cholic acid was injected. A total of 4700 mgm. was injected. A total of 300 cc. of urine was voided in four hours. Approximately, the equivalent of 1450 mgm. were excreted in the urine in three hours, 800 in the first hour.

Experiment 12. Dog 3, male collie, 18.6 kilos. Four per cent desoxycholate, and 16 per cent cholate solution were injected. The total quantities injected were 720 mgm. of desoxycholate and 3160 mgm. of cholic acid.

maximum of 75 mgm. per cent of desoxycholate hemolytic equivalents, or about 563 cholate equivalents took place when 300 mgm. per kilo of cholate were injected. The maximum urinary concentration reached 12 mgm. desoxycholate hemolytic equivalents or approximately 90 cholate equivalents when 170 mgm. of cholic acid per kilo were injected. This increased excretion continued for an hour after injection while the blood level was as before injection. When 300 mgm. per kilo were injected, the urinary increase lasted for 3 hours. In some experiments, definite diuresis accompanied the urinary rise in bile acid.

The injection of a mixture of desoxycholic and cholic acids produced changes in the blood and urine combining the results obtained with injections of the same doses of the individual bile acids.

The blood-urine bile acid ratio. The normal bile acid content of blood and urine assumes no constant relationship. The blood level may be slightly higher or vice versa. Following injection, however, a definite relationship is established, whenever the blood or urinary concentration is increased above the normal level.

Ratios between abnormal concentrations of bile acids in the blood and urine following the injection of bile acids may be calculated from data in table 1. A quotient is obtained by dividing the concentration of bile

acids in milligrams per cent of desoxycholate hemolytic equivalents in the blood by that in the urine. It is evident that with a single exception (expt. 5), the ratios following the injection of desoxycholic acid give quotients greater than unity (expt. 2, 1.3, 2.5; expt. 3, 2.5, 3.0, 3.0; expt. 4, 1.2, 2.2; expt. 6, 1.0, 3.0). The ratios following the injection of cholic acid all give quotients less than unity (expt. 8, 0.3; expt. 9, 0.1, 0.1; expt. 10, 0.05; expt. 11, 0.1, 0.1, 0.05).

COMMENT. The tested bile acids differ in the rate of clearance from the blood following intravenous injection. When cholic acid was injected, at twenty minutes and thereafter, the blood concentration of bile acids was as before the injection. A rise in the blood concentration first became manifest when 300 mgm. per kilo were injected. The injection of desoxycholic acid, on the other hand, was associated with a rise in the concentration of bile acids in the blood when 20 mgm. per kilo were injected. This increase was demonstrable for 2 hours after the injection. In some instances, the blood concentration was higher at 60 minutes than at 20 minutes. In some instances, a slight rise occurred at 240 minutes after the control level had been reached at 180 minutes. It cannot be stated at present whether these secondary rises depend on the injected bile acid intrinsically or upon bile acids derived from the liver.

The reactions to injection of the two acids also differ as to the amounts excreted in the urine. Though the first evidence of bile acid escape in the urine follows the same doses of both acids, cholic acid was eliminated three to twenty times more rapidly by the kidney than desoxycholic acid.

Significant relationships between blood level and urinary concentration may exist when either is elevated above normal. The blood may be elevated, and the urine normal. This occurred when 20 and 40 mgm. of desoxycholic acid per kilo were injected. The urinary concentration may be high, and the blood level normal. This occurred when amounts as high as 170 mgm. of cholic acid per kilo were injected. The blood and urinary concentrations may both be increased. This took place when doses of desoxycholic acid of 60 mgm. per kilo or more were injected. Under these conditions, with a single exception (expt. 5), the bile acid level of the

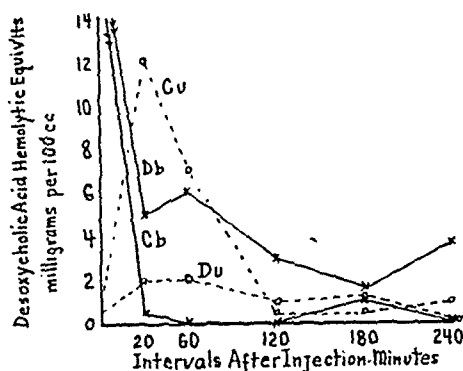


Fig. 1. Bile acid concentration of blood and urine at intervals after the rapid intravenous injection of desoxycholic and cholic acids.

Cu, o-----o Urine: 170 mgm. cholic acid injected; Cb, x-----x Blood: 170 mgm. cholic acid injected; Du, o-----o Urine: 40 mgm. desoxycholic acid injected. Db, x-----x Blood; 40 mgm. desoxycholic acid injected.

blood in desoxycholate hemolytic equivalents was higher than that in the urine. The observation is better noted 60 and 120 minutes after injection, than at 20 minutes when the distinction is less sharp.

The type of bile acid retained in the blood following the injection of desoxycholic acid cannot be positively identified as desoxycholic acid at present. It is probably not cholic acid since when cholic acid is injected directly, it promptly leaves the blood. The bile acid eliminated in the urine following the injection of cholic acid may be identified with cholic acid since studies have been reported, using colorimetric methods, which estimate cholic and glycocholic acids and not desoxycholic acid, which indicate a rapidly falling blood concentration and a rise in urinary concentration, at intervals beginning from the time of injection of glyco- and taurocholic acids (1).

Several hypotheses are advanced to account for the differences between the blood clearance rates and the urinary excretion of bile acids following the injection of the two bile acids. They cannot be attributed to renal injury alone since all bile acids probably produce some form of renal injury. Even the synthetic, relatively, non-toxic dehydrocholic acid, is nephrotoxic (7). The fact that when large doses of both bile acids are injected, they are followed by a continued renal excretion in as high a concentration as when they are injected individually, indicates that the retention in the blood cannot be attributed to greater renal injury by desoxycholic acid. Greater adsorption of desoxycholic acid to serum proteins cannot be accepted in explanation since desoxycholic acid has not been found to be bound to serum protein fractions in a greater degree, judging from the inhibitory effect of serum proteins on bile salt hemolysis (8). There may be a renal threshold for the excretion of desoxycholic acid and none for cholic acid. The singular capacity of desoxycholic acid to form addition or coördination compounds with various substances (9) may also possibly explain its delayed removal from the blood.

The major amounts of the injected bile acids are excreted in the bile. The amounts of bile acid lost in the urine following the injection of cholic acid is a small fraction of the entire dose, approximately 2.5 to 4 per cent computed on the basis that cholic acid is the bile acid excreted in the urine. This fraction may reach 30 per cent of the dose when 300 mgm. per kilo are injected.

When the bile acids are injected in restricted dosage, there is a tendency for bile acid retention in the blood following the injection of desoxycholic acid and for the excretion of bile acids in the urine following the injection of cholic acid (fig. 1). The bile acids occurring naturally in the blood and urine may differ fundamentally in type.

Experiments are in progress to determine whether under experimental and clinical conditions, liver injury and biliary stasis may produce sig-

nificant and characteristic changes in blood clearance or urinary excretion of bile acids.

SUMMARY

The blood clearance rates differ following the injection of desoxycholic and cholic acids. The blood is promptly cleared of cholic acid whereas the bile acid concentration of the blood remains elevated for 120 minutes after the injection of desoxycholic acid in restricted doses.

The urinary excretion of bile acid following the injection of cholic acid is several to many times greater than that following the injection of desoxycholic acid. The total amount of bile acid lost in the urine is usually a relatively small fraction of the injected dose in both instances.

With restricted doses of desoxycholic acid, bile acid may not be excreted in the urine, while the blood concentration may rise.

The blood urine bile acid concentration ratio, when the concentration in either fluid is increased above normal, following injection, may assume a specific and constant relationship depending on whether desoxycholic or cholic acid is injected.

REFERENCES

- (1) GREENE, C. H. AND A. M. SNELL. *J. Biol. Chem.* **78**: 691, 1928.
BOLLMAN, J. L. AND F. C. MANN. *This Journal* **116**: 214, 1936.
- (2) KÜHNE, W. *Virchow's Arch.* **14**: 310, 1858.
HOPPE-SEYLER, F. *Virchow's Arch.* **24**: 1, 1862.
HUPPERT, H. *Arch. Heilk.* **5**: 236, 1864.
- (3) LICHTMAN, S. S. *J. Biol. Chem.* **107**: 717, 1934.
- (4) TASHIRO, S. *Med. Bull. Univ. Cincinnati* **6**: 40, 1931.
- (5) WIELAND, H. AND T. HILDEBRAND. *Arch. exp. Path. und Pharmacol.* **85**: 199, 1920; **86**: 79, 92, 1920.
- (6) LICHTMAN, S. S. *J. Lab. Clin. Med.* **22**: 204, 1936.
- (7) STEWART, H. L. AND A. CANTAROW. *Arch. Path.* **20**: 866, 1935.
- (8) LICHTMAN, S. S. Unpublished observations.
- (9) WIELAND, H. AND H. SORGE. *Ztschr. f. physiol. Chem.* **97**: 1, 1916.

VAGINAL AND UTERINE GRAFTS IN THE RAT AS INDICATORS OF THE PRODUCTION OF OESTRIN

CARROLL A. PFEIFFER¹

*From the Barbara Henry Research Laboratory of the New York Hospital and the
Department of Medicine, Cornell University Medical College, New York*

Received for publication July 28, 1936

Recent investigation (Pfeiffer, 1936a) indicates a non-cyclic action of the male hypophysis in the rat and therefore a constant production of the sex hormones. Ovarian grafts in the male, whether normal or castrated, have good follicular development (Goodman, 1934) but luteinization does not occur unless gonadotropic hormones are injected. These same conditions are true of the ovaries of females with masculinized hypophyses (Pfeiffer, 1936a). However, in the latter animals it is much easier to analyze the hormonal state of both the hypophysis and the ovary. The masculinized hypophysis is not able to release luteinizing hormone in sufficient amount to cause luteinization but the injection of it produces all the cyclic action of the ovary (Witschi and Pfeiffer, 1935). There is a balance between the oestrin produced by the ovary and the follicle-stimulating hormone of the hypophysis which results in a non-cyclic and constant production of oestrin that is above the requirement of the vagina for oestrus but below that necessary for full oestrus of the uterus. Since Martins (1932) concluded that cycles were present in the vaginal grafts in castrated males carrying ovarian grafts, it is necessary to investigate more fully the production of oestrin by an ovarian graft in the male. The only adequate method of determining the cyclic production of oestrin in the rat is by the cell proliferation of the vaginal epithelium. However, since the uterus requires a higher level of oestrin, it would appear to be advisable to graft both vaginal epithelium and uterine endometrium into the various animals to be tested.

METHODS. Albino rats from a single commercial strain were used in all of these experiments. The ovary was placed in the right eye after the technique of Goodman (1934), the uterine endometrium and vaginal epithelium in the left eye after the technique of Markee (1929), and the vaginal grafts under the skin after the technique of Martins (1932). Host and donor were 85 days of age. All operations were performed under deep ether anesthesia. The grafts were examined daily under a binocular

¹ Henry Research Fellow in Medicine, New York Hospital.

dissecting microscope ($\times 20$); but it was necessary to prepare histological sections in order to study in detail the effects on the vaginal epithelium. All grafts, gonads, and accessory sexual organs were removed at autopsy (after 45 days), sectioned at 10μ , and stained with cosin and Delafield's hematoxylin.

RESULTS. 1. *Uterine and vaginal grafts in normal females.* Seven females received uterine endometrium and seven received vaginal epithelium in the anterior chamber of the eye, in order to determine the re-

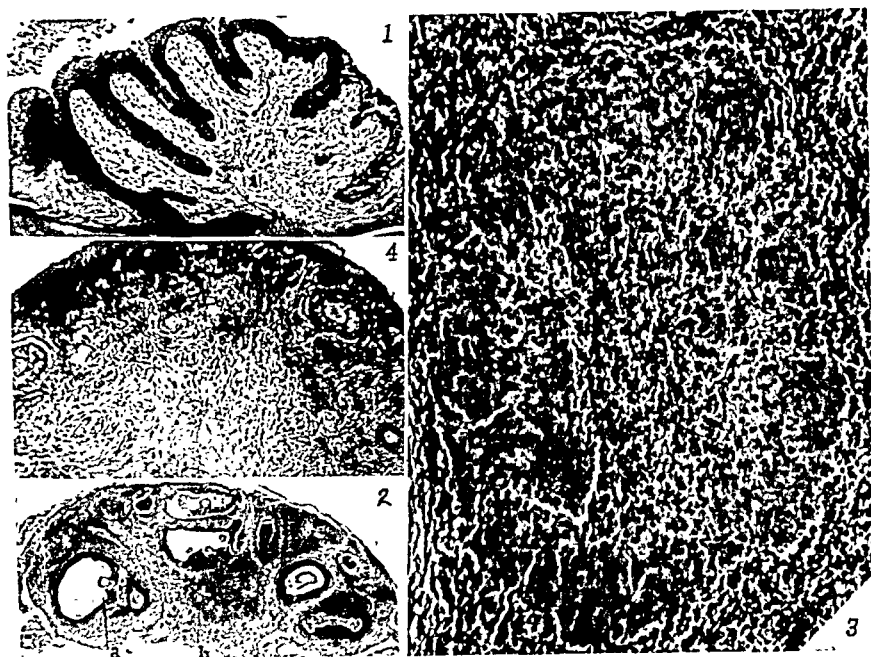


Fig. 1. Photomicrograph of a cross section of the vaginal epithelium graft in the male castrate rat showing the keratinized epithelium. $\times 45$.

Fig. 2. Cross section of an ovarian graft in the castrate male. a. Normal graafian follicle. b. Corpus luteum. $\times 15$.

Fig. 3. The corpus luteum of figure 2 at a higher magnification. Note the characteristic lutein cells. $\times 75$.

Fig. 4. Cross section through an ovarian graft in the non-castrate male showing several primary, but no maturing, follicles. $\times 45$.

sponse to the oestrin produced by the normal ovary. The grafts (homotransplants) remained vitalized in all but one animal in each group. There was a distinct hyperemia of the uterine endometrial graft corresponding to the full oestral change in the intact uterus, but the characteristic alternate blanching and blushing of the endometrium in other species (Markee, 1929, 1931, 1932a and 1932b) was not observed here. Histological sections of the vaginal epithelial grafts made during the various stages of the normal cycle demonstrated the same changes in the graft

as in the intact vagina. Since the uterine endometrial and vaginal epithelial grafts responded to oestrin produced by the ovary in the same manner as if they were in their respective intact positions, they could be safely used as a measure of oestrin production.

2. *Vaginal epithelial grafts in males bearing ovarian grafts.* Seven normal and six castrated males received $\frac{1}{3}$ of an ovary in the anterior chamber of the left eye and a small piece of vaginal epithelium in the right. As in the case of the normal females (section 1) nothing could be ascertained as to the cell proliferation in the intact vaginal graft. It was found upon sectioning (after 45 days) that the vaginal epithelium was thick and keratinized in the castrates (fig. 1), while in the non-castrates the epithelium was only a few layers in thickness and resembled the dioestrous stage of the intact epithelium.

Histological study of the ovarian grafts from the castrate group showed that numerous follicles were to be found in all stages of development up to the preovulatory stage (fig. 2). In two grafts several degenerating corpora lutea were found, one of which still showed the fairly typical staining reactions of the normally functioning corpus luteum (fig. 3). In the non-castrates the ovarian grafts contained only a few primary follicles (fig. 4) and in one case only a single follicle. The male accessory sexual organs demonstrated a suppression of the male hormone in these non-castrates as previously described by Pfeiffer (1936b).

The absence of keratinization of the vaginal grafts in the non-castrated males may be explained by the condition of the ovarian grafts which obviously produced very little oestrin. However, in the castrate, where the ovary was in equilibrium with the male hypophysis, the oestrin level was high enough to cause keratinization of the vaginal epithelium. This indicated a full oestral condition as far as the vagina was concerned. There was no reaction that could be interpreted as pointing toward a cyclic production of oestrin. Therefore, in order to test whether there was a cyclic reaction of the vaginal graft in the male, smears were followed on a segment of vagina placed under the skin.

3. *Males with vaginal grafts under the skin.* Twelve male rats received vaginal grafts under the skin according to the technique of Martins (1932). All received ovarian grafts and six were castrated at the same operation. The castrated group received a 21 day old ovary in the right eye and the normal group received an ovary in the right testis.

In the normal group the vaginal grafts did not all survive. It was thought unwise to inject oestrin (as suggested by Martins, 1932) to establish the graft as that would suppress the hypophysis and indirectly injure the ovarian graft. Neither was it desirable to inject gonadotropic substances as they would, at least for a time, influence the reaction of the ovary and cover up the true action of the ovarian graft. Only three vagi-

nal grafts proved successful enough so that daily smears could be made. In these animals there was continuous musification. It had been shown by Myer and Allen (1931 and 1932) that musification could be produced by the action of less than one half the oestrin required for cornification.

In the castrate group five of the vaginal grafts were sufficiently successful to allow daily smears to be followed for at least a month. In all of these there was a continuous oestrus except for a few brief, rather irregular, interruptions. The cornified cell stage lasted from 15 to 25 days and was interrupted only by a short dioestral smear or leucocytic stage of about 36 hours. This was not interpreted as a cyclical phenomenon.

The histological appearance of the ovarian grafts was similar to that found in the group in which the vaginal epithelium was placed in the eye. As in the other experiments the difference in the reaction of the vaginal epithelium in the castrate and non-castrate groups was undoubtedly due to the physiological condition of the ovarian graft.

4. *Uterine endometrial grafts in males bearing ovarian grafts.* Six normal and six castrated males received ovaries in the left eye. At the same operation a small piece of uterine endometrium was placed in the right eye. Two of the non-castrate animals were autopsied at 25 and 35 days respectively because of a respiratory infection, the so-called "snuffles." All others were autopsied after 45 days.

Daily observations failed to show any hyperemic condition such as found in the female at oestrus. On histological section the endometrium resembled that found during dioestrus in the intact position. There was no difference in reaction between the castrate and the non-castrate groups except that the animals with "snuffles" infection showed very degenerative grafts. The ovarian grafts were the same as those in the previous experiment except one case, in the non-castrate group, which contained many developing follicles. As this single host showed no deficiency of the male hormone, a common factor was indicated as the cause of suppression of both the testis and ovarian graft. It was clear, however, that the amount of oestrin released by the ovary in the male, whether castrated or normal, was below that required for the oestrus of the uterus.

DISCUSSION. The above experiments demonstrate that the ovarian graft in the male releases oestrin in a non-cyclical manner and at a fairly constant level. However, the fact that there is no alternate blanching and blushing of the uterine endometrium in the eye of the rat is in marked contrast to that observed by Markee (1929, 1931, 1932a and 1932b) for the rabbit, guinea pig and monkey. There are two possible explanations for the absence of this phenomenon: 1, it may be due to a pressure factor, as the anterior chamber of the eye is small, or 2, there may be a species difference. There is some evidence (unpublished work) that both are contributing factors. An explanation of the absence of the alternate

blanching and blushing is not essential to the interpretation of the results of these experiments. There is a characteristic hyperemia of the endometrial graft during the oestrus of the normal female which never occurs in the male. This and the constant cornification of the vaginal epithelium demonstrate that the oestrin level, as in the females with masculinized hypophyses, is above that required for the vaginal cornification but below that necessary for oestrus in the uterus. The absolute parallelism between the physiological action of oestrin in the female with the masculinized hypophysis and that produced by the ovarian graft in the male, indicates that the release of hormone from their hypophyses is also similar and consists of only follicle stimulating hormone.

The results of daily smears of the vaginal grafts under the skin are essentially the same as those of Martins (1932). He observed cornified cells for 6 to 15 days, leucocytes for 1 to 2 days, and then another long oestrous phase. The longer cornified cell stage in our investigation, due to the eye being a better transplantation site for the ovary, demonstrates that it is really a state of continuous oestrus. The break in the continuous oestrus is due to transplantation factors (Pfeiffer, 1934) and is not an indication of a cyclic action of the ovarian graft or the hypophysis of the male.

Since there is no evidence of cyclic release of oestrin, it is of interest that corpora lutea are sometimes present 45 days after transplantation of the mature ovary into castrate males. It is likely that they have persisted from the time of transplantation as Smith (1930) has shown that corpora lutea may persist for a long time in the ovary of the hypophysectomized female. However, Pfeiffer (1936a) has definitely shown that unless the testis has been present in the pre-pubertal stage, corpora lutea regularly form in the grafted ovary in the male. Therefore, the time of castration is important and in this case, as well as the few that have been reported (Moore, 1919, and Wang, Richter and Guttmacher, 1925) the corpora lutea may have formed after transplantation as Evans et al. (1935) have shown that some luteinizing hormone is present in the hypophysis of the castrate male. The male hypophysis does not normally release luteinizing hormone at least in amounts sufficient for luteinization, as the ovarian graft in the male (normal or castrate) shows only follicular development.

The observations that the ovarian grafts are usually degenerative in character and the male accessory sexual organs are suppressed in the non-castrate males may be taken as an indication of mutual antagonism between the testis and ovary. However, present investigation (in progress) suggests that it may be due to the toxic factors introduced by the so called "snuffles" infection. The demonstration by Myers and Allen (1931, 1932) that musification of the vagina is produced by suboestral amounts of oestrin explains the musification observed in the non-castrate group where the ovarian grafts were suppressed.

SUMMARY AND CONCLUSIONS

1. Uterine endometrium and vaginal epithelium were successfully transplanted into the anterior eye chamber of the rat. Hyperemia of the uterine endometrial graft during oestrus enabled the cycles of the normal female to be followed by this method. However, cycles could be demonstrated in the grafted vaginal epithelium only upon histological examination.

2. In male rats, either normal or castrated, the uterine endometrial grafts gave no evidence of complete oestral reaction to the oestrin produced by an ovarian graft. The vaginal epithelium was continually cornified in the castrate group but in the non-castrate group the suppressed ovaries were not able to produce more than enough oestrin to cause musification.

3. The uterine endometrial and vaginal epithelial grafts demonstrated cyclic function of the ovary in the normal female but not of the ovarian graft in the male. This lends further evidence that the hormone produced or released from the male hypophysis is non-cyclic and that the female hypophysis is essential for the cyclic function of the ovary.

REFERENCES

- EVANS, H. M., M. E. SIMPSON AND R. I. PENCHARZ. *Proc. Soc. Exper. Biol. and Med.* **32**: 1048, 1935.
- GOODMAN, L. *Anat. Rec.* **59**: 223, 1934.
- MARKEE, J. E. *Am. J. Obst. Gynec.* **17**: 205, 1929.
Anat. Rec. **48**: Supplement, 28, 1931.
This Journal **100**: 32, 1932a.
This Journal **100**: 374, 1932b.
- MARTINS, T. *Compt. rend. Soc. de Biol.* **109**: 134, 1932.
- MYER, R. K. AND W. M. ALLEN. *Anat. Rec.* **51**: Supplement 21, 1931.
Science N.S. **76**: 111, 1932.
- MOORE, C. R. *J. Exper. Zool.* **28**: 137, 1919.
- PFEIFFER, C. A. *Proc. Soc. Exper. Biol. and Med.* **31**: 479, 1934.
Am. J. Anat. **58**: 195, 1936a.
Anat. Rec. **65**: 213, 1936b.
- SMITH, P. E. *Am. J. Anat.* **45**: 205, 1930.
- WANG, G. H., C. P. RICHTER AND A. F. GUTTMACHER. *This Journal* **73**: 581, 1925.
- WITSCHI, E. AND C. A. PFEIFFER. *Anat. Rec.* **64**: 85, 1935.

THE BIOASSAY OF ADRENAL CORTICAL EXTRACTS

A DIRECT COMPARISON OF RAT AND DOG UNITS

GEORGE F. CARTLAND AND MARVIN H. KUIZENGA

From the Research Laboratories, The Upjohn Company, Kalamazoo, Michigan

Received for publication July 29, 1936

Harrop, Pfiffner, Weinstein and Swingle (1) and Pfiffner, Swingle and Vars (2) have elaborated a dog method of assay and have defined the dog unit as the minimum daily per kilogram dose of adrenal cortical hormone necessary to maintain normal physiological conditions in the adrenalectomized dog for a period of 7 to 10 days, the two criteria of normal physiologic conditions being maintenance of body weight and blood level of non-protein nitrogen (or urea). This method has been used extensively by research workers in this field and is generally regarded as the best available method for the bioassay of adrenal cortical extracts.

Adrenalectomized rats have also been used. The fact that rats rarely survive adrenalectomy if the glands are carefully and completely removed was demonstrated by Pencharz, Olmstead and Gerogossintz (3) and Freed, Brownfield and Evans (4). Kutz (5) has studied the use of adrenalectomized rats and has defined the rat unit as the minimum daily dose of cortical hormone which will protect, for at least 20 days, 50 per cent of a group of animals adrenalectomized at 28 days of age, the extract being administered subcutaneously twice daily.

The suitability of young adrenalectomized rats for assaying adrenal cortex extracts has been considered further by Firor and Grollman (6), Gaunt (7, 8), Schultzer (9) and Cleghorn and co-workers (10). A rat method of assay was used by Grollman and Firor (11) for evaluating adrenal cortical extracts.

A review of the literature indicates that there is a great difference of opinion regarding the suitability of adrenalectomized rats for testing adrenal cortical extracts. Most of the disagreement seems to center in the number of indefinite survivals encountered among untreated controls and after cessation of treatment with hormone. If the adrenalectomized rat is to be regarded as a satisfactory animal for assay of adrenal cortical extracts, there should be no large incidence of indefinite survivals among controls, and the rats successfully maintained by hormone therapy should die in the normal time after cessation of injections.

The rat method is less time-consuming than the dog method, which constitutes a decided advantage in following the hormone distribution in connection with fractionation studies. However, we have noted no reports in the literature where the rat and dog assays have been run in parallel, and it appears that this is necessary before any high degree of confidence can be placed in the rat method. The present study is concerned with a direct parallel comparison of the rat and dog methods applied to the same adrenal cortical extracts for the purpose of establishing the relative values of the dog and rat units respectively.

EXPERIMENTAL. The adrenal cortical extracts used in these studies were prepared by a method developed in this laboratory, which will be described in a separate report. The extracts were made up so that 40 grams of fresh beef adrenal glands were represented in each cubic centimeter. These extracts contained from 0.6 to 1.0 mgm. of gland extractives per cubic centimeter and were substantially free of epinephrin and other toxic impurities. The solutions assayed by the dog blood pressure method showed less than 1 part of epinephrin per 400,000.

The dog method. This method has been followed throughout as described by Pfiffner, Swingle and Vars (2), except that the total non-protein nitrogen increase was followed, instead of urea nitrogen. Non-protein nitrogen was determined by the method of Koch and McMeekin (12) on the trichloroacetic acid filtrate of the blood. The technique for removal of adrenals from dogs, described by Banting and Gairns (13) was followed.

The rat method. Young male rats of Wistar strain, 4 weeks old and weighing 50 to 60 grams, were used. The rat rooms were kept at 75 to 80°F. The diet consisted of Purina Dog Chow upon which the rats show excellent growth without need of dietary supplements. The analysis given by the manufacturers is sodium 0.67 per cent, chloride 0.68 per cent.

The rats were anesthetized with sodium amytal 60 mgm. per kilo intraperitoneally which was supplemented with a little ether when necessary. The operative field was shaved and washed with 70 per cent alcohol. An oblique incision was made on the right side in the lumbar region, and the muscles divided parallel and posterior to the lowermost rib. The right kidney can be easily pushed forward through the incision. By gently tilting up the anterior pole of the kidney, the adrenal gland with its pedicle was clearly exposed. The pedicle of the adrenal was grasped anteriorly of the gland by means of forceps and the pedicle cut with scissors just anterior to the forceps. The gland and the fatty tissue in which it is embedded were then lifted upward and backward. In this way one-third to one-half of the capsule of the kidney is removed which insures a clean removal of all fatty tissue around the adrenal gland. By grasping the cut edge of the muscle and gently lifting it, the kidney can easily be made to

drop back into its normal position. The muscle and skin were brought together with a single silk suture. The same procedure was used for removal of the left gland except that the incision was made about 1 cm. more posteriorly and just dorsal to the upper part of the spleen. Each rat was wrapped in cotton from which he could extricate himself when no longer anesthetized. By means of this technique, forty to fifty can easily be adrenalectomized in six to seven hours by one operator. The instruments and silk sutures are kept in 70 per cent alcohol and the operator's hands washed with this solution. On the third or fourth day, when the wounds are already tightly closed, the sutures are removed.

The hormone solutions were administered by single subcutaneous daily injections beginning on the day of operation and continued for 20 days. Not less than 5 uninjected controls were used with every series, the usual

TABLE 1
Survival period after adrenalectomy in rats

GROUP	NUMBER OF RATS OPERATED	AVERAGE SURVIVAL AFTER OPERATION	NUMBER SHOWING INDEFINITE SURVIVAL
I. Controls not injected.....	192	days 6.88	5
II. Injected for 20 days with active ex- tracts.....	240	27.21*	8
III. Injected with extracts which were prac- tically inactive.....	157	7.27	4
IV. Injected with extracts of low activity...	86	16.24	2
Total.....	675		19

* These rats survived on an average 7.21 days after discontinuing hormone injections.

procedure being to inject parallel groups of 5 rats each with 0.1, 0.2, and 0.3 cc. respectively of hormone solution.

RESULTS. Up to the present time 675 rats have been adrenalectomized by the above described technique. This number comprises four groups and the data concerning their survivals are given in table 1. In the control group of 192 rats the average survival after operation was 6.88 days. In group II, the 240 rats which received maintenance dosages of extract for 20 days showed an average survival of 7.21 days after discontinuing injections. In group III, the 157 rats which were injected with inactive extracts showed an average survival of 7.27 days after operation which is essentially the same as that of the control group. The 86 rats in group IV received sufficient doses of extract to definitely increase their survival period over that of controls but not sufficient to maintain them for 20 days, the routine period of injection.

Whenever an adrenalectomized rat survived more than 15 days after operation or after cessation of injections, it was considered as an "indefinite survival" and search was made on autopsy for residual adrenal cortex tissue. Such animals must be excluded in calculating rat units, although they have been included in table 1. Of the total 675 rats listed in table 1, only 19 showed indefinite survival, and in 14 of these, adrenal rests were found on autopsy to account for the prolonged survival. Thus, in our experience the mortality in rats after adrenalectomy is 97 per cent and

TABLE 2
Adrenal cortical extracts assayed in rat units

EXTRACT NUMBER	DAILY DOSE (1 CC. REPRESENTS 40 GRAMS GLAND)	NUMBER OF RATS INJECTED	NUMBER OF RATS SURVIVING ON 20TH DAY	AVERAGE GROWTH IN 20 DAYS	AVERAGE SURVIVAL AFTER LAST INJECTION	RAT UNITS PER CUBIC CENTIMETER OF EXTRACT
	<i>cc.</i>			<i>grams</i>	<i>days</i>	
245 MHK-2	0.3	5	5	23	5	$\left. \begin{array}{l} >6.6 \\ <10 \end{array} \right\} 8.3$
	0.2	5	5	24	7	
	0.15	5	5	30	8	
	0.10	5	4	15	6.5	
	0.05	6	4	8.5	4.5	
292 MHK-2	0.3	5	5	22	4.0	$\left. \begin{array}{l} >3.3 \\ <5.0 \end{array} \right\} 4.1$
	0.2	6	5	13	5.4	
	0.1	6	0			
185 MHK-2	0.3	5	5	37	9.3	5.0
	0.2	5	5	20	5.2	
51 MHK-5	0.3	5	4	18	4.0	5.0
	0.2	5	5	21	5.4	
	0.1	5	0	5		
230 MHK-2	0.3	5	4	24	6.0	$\left. \begin{array}{l} >3.3 \\ <5.0 \end{array} \right\} 4.1$
	0.2	5	4	12	4.3	
11 MHK-5	0.3	7	6	25	5.0	$\left. \begin{array}{l} >3.3 \\ <5.0 \end{array} \right\} 4.1$
	0.2	6	4	20	4.5	

the average survival of those rats which are adequately maintained for 20 days by daily injections of hormone is practically the same after cessation of injections as the average survival period of the uninjected controls.

The next step was to define a rat unit in such a way as to permit a quantitative estimation of the hormone content of various adrenal cortical extracts. After a series of preliminary experiments, the rat unit was defined as the minimum daily dose of hormone which, administered by single subcutaneous injection daily for 20 days to 4 weeks old male rats

weighing 50 to 60 grams, is sufficient to protect at least 80 per cent of the rats and produce an average growth of at least 20 grams per rat for the 20 day period. The survival and growth data together with the calculated rat units are given in table 2 for six active extracts assayed by the rat method.

The results in table 2 show that at the higher dosages of extract the growth is not proportional to dosage, which is in agreement with the criticism of the rat method given by Cleghorn and co-workers (10) in a recent report. Consequently, growth alone is not a satisfactory basis of assay. However, it is apparent from the data in table 2 that there is for each extract a certain minimum dose at which not less than 80 per cent of the rats survive and the average growth for the 20 day injection period is not less than 20 grams per rat. This has been selected as the basis of the rat unit. This dose for extract 245 MHK-2 is greater than 0.1 cc. and less than 0.15 cc.; for 292 MHK-2 it is greater than 0.2 and less than 0.3 cc. per rat daily. For 185 MHK-2 the average growth on 0.2 cc. was 20 grams, consequently this was taken as the unit dose. If the dosage is lowered below the minimum which will maintain 80 per cent and bring about an average growth of 20 grams in 20 days, the rate of growth falls off rapidly.

A unit could be based solely upon survival of at least 80 per cent of the rats, and such a unit would be approximately 25 per cent smaller than the one based on survival plus growth. However, we have chosen the larger unit because it more nearly approximates a physiological maintenance unit for the adrenalectomized rat. For convenience we have adopted a single daily subcutaneous injection, although we realize that a divided dosage may give a more effective utilization of the hormone. Also we have some preliminary experiments which indicate that when the hormone is administered in the drinking water the growth may be more proportional to dosage.

Comparison of rat and dog methods of assay. As a check on the value of the rat method as described above, a number of extracts were assayed in parallel by the rat and dog methods, using the dog method as described by Harrop and co-workers (1). The results obtained with eight preparations are given in table 3. As previously mentioned, all extracts were made up so that each cubic centimeter represented 40 grams of whole beef adrenal glands.

The ratio of dog units to rat units varies from 17.8 to 25.4 with an average of 21.7 for the extracts compared. The results given in table 3 indicate that the quantitative estimation of activity by the rat method follows very closely the results obtained by the dog method, although the rat's requirements are much higher thus giving a larger unit. Preparation 245 MHK-2 which gave the highest rat assay also gave the highest dog assay. Preparation 122 MHK-2 gave the lowest dog assay and also the lowest

TABLE 3
Comparison of rat and dog units

EXTRACT NUMBER	RAT UNITS PER CUBIC CENTIMETER	DOG UNITS PER CUBIC CENTIMETER	RATIO $\frac{\text{DOG UNITS}}{\text{RAT UNITS}}$
102 MHK-2	3.3	Dog 1, $\left. \begin{array}{l} >40 \\ <80 \end{array} \right\}$ 60	$\frac{60}{3.3} = 18.2$
136 MHK-2	$\left. \begin{array}{l} >3.3 \\ <5.0 \end{array} \right\}$ 4.1	Dog 1, $\left. \begin{array}{l} >66 \\ <133 \end{array} \right\}$ 100	$\frac{100}{4.1} = 24.4$
230 MHK-2	$\left. \begin{array}{l} >3.3 \\ <5.0 \end{array} \right\}$ 4.1	Dog 1, $\left. \begin{array}{l} >68 \\ <136 \end{array} \right\}$ 102	$\frac{102}{4.1} = 24.8$
245 MHK-2	$\left. \begin{array}{l} >6.6 \\ <10 \end{array} \right\}$ 8.3	Dog 1, $\left. \begin{array}{l} >138 \\ <276 \end{array} \right\}$ 207 Dog 4, $\left. \begin{array}{l} >108 \\ <216 \end{array} \right\}$ 162 Dog 5, $\left. \begin{array}{l} >73 \\ <146 \end{array} \right\}$ 110 Average 160	$\frac{160}{8.3} = 19.3$
292 MHK-2	$\left. \begin{array}{l} >3.3 \\ <5.0 \end{array} \right\}$ 4.1	Dog 4, $\left. \begin{array}{l} >56 \\ <112 \end{array} \right\}$ 84 Dog 7, $\left. \begin{array}{l} >42 \\ <84 \end{array} \right\}$ 63 Average 73	$\frac{73}{4.1} = 17.8$
14 MHK-5	$\left. \begin{array}{l} >3.3 \\ <5.0 \end{array} \right\}$ 4.1	Dog 4, $\left. \begin{array}{l} >116 \\ <232 \end{array} \right\}$ 174 Dog 6, $\left. \begin{array}{l} >40 \\ <80 \end{array} \right\}$ 60 Dog 8, $\left. \begin{array}{l} >50 \\ <100 \end{array} \right\}$ 75 Average 104	$\frac{104}{4.1} = 25.4$
51 MHK-5	5.0	Dog 4, $\left. \begin{array}{l} >62 \\ <124 \end{array} \right\}$ 93 Dog 8, $\left. \begin{array}{l} >84 \\ <168 \end{array} \right\}$ 126 Average 109	$\frac{109}{5.0} = 21.8$
122 MHK-2	<2.0	Dog 1, $\left. \begin{array}{l} >15 \\ <30 \end{array} \right\}$ 22.5	

rat assay, although for this extract the lower limit in rat units was not established and consequently the ratio of dog to rat units cannot be given.

It is now well established that the survival period and clinical condition of adrenalectomized rats and dogs can be improved by the addition of certain supplements to the diet, particularly sodium chloride. Consequently in using either the rat or dog method it is very important to control the diet rigidly if quantitative estimations are desired. However, we have not been able to maintain indefinitely either completely adrenalectomized rats or dogs by dietary supplements alone without the administration of adrenal cortical hormone.

It is interesting here to compare the hormone requirements of the adrenalectomized dog and rat when calculated on a per kilogram basis. The seven preparations in table 3 for which ratios of dog to rat units have been determined show an average of 101 dog units per cubic centimeter and 4.7 rat units per cubic centimeter. The dog unit represents the physiological maintenance dose per kilogram of dog. However, the rat unit represents the physiological maintenance dose per rat, which during the 20 day period of injection and growth, weighs on an average approximately 65 grams. When both of these maintenance doses are calculated on a per kilogram basis, the dog is found to require 0.01 cc. and the rat $\frac{1}{4.7} \times \frac{1000}{65} = 3.27$ cc. of extract per kilogram. These doses represent 0.4 and 131 grams respectively of fresh beef adrenal glands. Thus, the young growing rat after adrenalectomy requires per unit of body weight more than 300 times as much adrenal cortical hormone as does the adult adrenalectomized dog.

SUMMARY

A rat method of assay has been used in parallel with the dog method in testing a number of adrenal cortical extracts. The rat unit has been defined as the minimum daily dose of hormone which, administered by single subcutaneous injection daily for 20 days to four weeks old male rats weighing 50 to 60 grams, is sufficient to protect at least 80 per cent of the rats and produce an average growth of at least 20 grams per rat for the 20 day period. The dog unit is the same as that defined by Pffner, Swingle and Vars (2).

The rat unit is approximately 22 times as large as the dog unit. On a per kilogram basis the young, growing rat after adrenalectomy requires more than 300 times as much adrenal cortical hormone as does the adult adrenalectomized dog.

Under the experimental conditions described, in a group of 675 rats, a mortality of 97 per cent has been observed in rats following adrenalectomy.

tomy when no hormone was given or after hormone injections were discontinued. Indefinite survival in the other 3 per cent has been accounted for in most cases by finding residual adrenal cortex tissue at autopsy.

The average survival period observed in a control group of 192 adrenalectomized rats receiving no hormone injections was 6.8 days. In a group of 240 adrenalectomized rats which were maintained by 20 daily injections with cortical extracts, the average survival after cessation of injections was 7.2 days or approximately the same as that of the controls.

The results indicate that the rat method shows satisfactory agreement with the dog method in estimating the hormone content of adrenal cortical extracts.

REFERENCES

- (1) HARROP, G. A., J. J. PFIFFNER, A. WEINSTEIN AND W. W. SWINGLE. *Sci.* **73**: 683, 1931; *Proc. Soc. Exper. Biol. Med.* **29**: 449, 1932.
- (2) PFIFFNER, J. J., W. W. SWINGLE AND H. M. VARS. *J. Biol. Chem.* **104**: 701, 1934.
- (3) PENCHARZ, R. I., J. M. D. OLMSTEAD AND G. GEROGSSINTZ. *Sci.* **72**: 175, 1930.
- (4) FREED, S. C., B. BROWNFIELD AND H. M. EVANS. *Proc. Soc. Exper. Biol. Med.* **29**: 1, 1931.
- (5) KUTZ, R. L. *Proc. Soc. Exper. Biol. Med.* **29**: 91, 1931.
- (6) FIROZ, W. M. AND A. GROLLMAN. *This Journal* **103**: 686, 1933.
- (7) GAUNT, R. *This Journal* **103**: 494, 1933.
- (8) GAUNT, R. AND J. H. GAUNT. *Proc. Soc. Exper. Biol. Med.* **31**: 490, 1934.
- (9) SCHULTZER, P. *J. Physiol.* **84**: 70, 1935.
- (10) CLEGHORN, R. A., S. M. M. CLEGHORN, M. G. FORSTER AND G. A. McVICAR. *J. Physiol.* **86**: 229, 1936.
- (11) GROLLMAN, A. AND W. M. FIROZ. *J. Biol. Chem.* **100**: 429, 1933.
- (12) KOCH, F. C. AND T. L. McMEEKIN. *J. Am. Chem. Soc.* **46**: 2066, 1924.
- (13) BANTING, F. G. AND S. GAIRNS. *This Journal* **77**: 100, 1926.

THE POTENTIAL ANALYSIS OF A PACEMAKER MECHANISM IN LIMULUS POLYPHEMUS

PETER HEINBECKER

*From the Department of Surgery, Washington University School of Medicine, St.
Louis, Missouri*

Received for publication July 30, 1936

The nature and properties of fiber potentials have been determined for the peripheral somatic and autonomic nervous systems in considerable detail (Erlanger, Bishop and Gasser, 1926; Heinbecker, 1929). Many correlations between potential form and bodily function have been established (Heinbecker, Bishop and O'Leary, 1936). Before corresponding correlations can be established for the central nervous system it is necessary that the characteristics of ganglion cell potentials be more specifically recognizable. The difficulty of solving this problem rests primarily on the impossibility of eliminating or identifying axon potentials in records of both axon and cell potentials. Indeed, until such times when potentials can be recorded from single cell bodies with shielded micro-electrodes, inferences concerning these cell potentials must be drawn from records made with leads subject to the influence of groups of cells and fibers. Evidence of this latter type has been advanced by Fröhlich (1913), by Adrian and Mathews (1928), by Adrian (1931), by Hartline and Graham (1932) and by Eccles (1935). Their findings suggest that the activity potential of certain ganglion cells acting alone, or as a small associated group, is sustained for a much longer time period than is that of their axons. Bishop (1936) has not been able to assign the long potentials found by Eccles to cell bodies of the superior cervical ganglion of the rabbit. The writer has never seen them in studies on the superior cervical sympathetic ganglion of the turtle. In studies of the mammalian cortex the long potentials generally have been regarded as summation phenomena. It seems highly probable that different types of ganglion cells vary considerably in the nature of their potentials so that further results from investigations of ganglion cells possessing various functions and in various species will have to be available before any generalizations as to their nature are advisable.

Previous studies on the ganglionated median cardiac nerve of *Limulus polyphemus* (Heinbecker, 1933) indicated that this preparation offered certain advantages as material for the study of ganglion cell potentials. The nerve is completely separable from the heart, permitting its investiga-

tion without the complications of muscle potentials which have so far masked all nerve potentials in investigations of the vertebrate heart. The cardiac ganglionic chain of *Limulus* possesses relatively few cell types and some of the cells are very large. The different cell types are differently distributed throughout the preparation so that it is possible by sec-

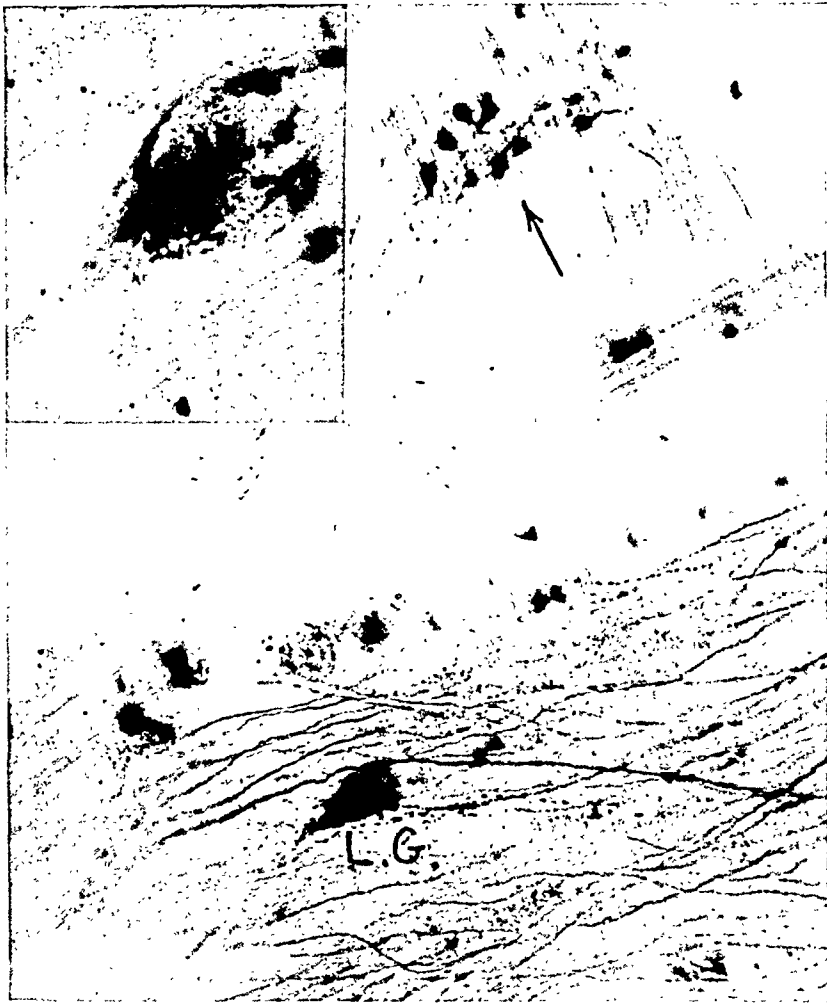


Fig. 1. Photomicrograph of methylene blue preparation of median cardiac nerve of *Limulus polyphemus* to show large (L.G.) and small (marked with arrow) ganglion cells. Magnification $\times 66$. Insert shows a large unipolar ganglion cell magnification $\times 220$.

tioning to obtain portions of the chain in which some of the types are lacking. Such sectioning of the preparation eliminates spontaneous activity only in the anterior three and the posterior cardiomerer. This is in itself convenient and leads to the inference that cells of the type possessed by these four cardiomerer do not by themselves display the spontaneous

activity shown by certain other cell types. While portions of the ganglionic chain display an intrinsic activity they can also be artificially excited.

The median cardiac nerve contains many non-myelinated fibers of various diameters which in some instances extend nearly throughout the length of the structure. These can be artificially stimulated and their potentials investigated without interference from ganglion cell potentials. In this report there are presented the results of an investigation of the potentials of the median cardiac nerve, particularly as they seem to offer a means of differentiation between fiber and ganglion cell potentials. The results seem to indicate that there is at least one type of ganglion cell which alone or in association with a small number of cells of a second type produces a potential having a duration longer than that of the single axons. This sustained potential of the ganglion cells is associated with repetitive responses of the axons. The effect of certain drugs, such as strychnine, adrenalin and cocaine, on the sustained potential has been the subject of investigation.

For an interpretation and understanding of the result of the potential analyses it is essential to present a brief résumé of certain of the cytological studies of the median cardiac nerve. Reference is made to the work of Patten (1912). It has been our privilege to study much of his original material.¹ We have also made preparations according to the method of Bodian (1936).² The ganglion cells of the median cardiac nerve are of several types (fig. 1). There are first giant unipolar cells measuring approximately 140 x 100 microns in methylene blue preparations. In the silver preparations there is considerable shrinkage, the average values for the greatest diameters ranging from 80 to 110 microns. These cells are not found in all cardiomeres, being absent in the first three and the last cardiomeres. Their axons extend in many instances almost the entire length of the cord. All their collaterals appear finally to pass out of the cord into the general nerve plexus of the heart. Scattered among the giant unipolar cells there are a few typical bipolar cells. These are somewhat smaller than the unipolar cells having an average diameter which is less by some 15 to 20 microns. A third type of cell is multipolar and measures from 30 to 60 microns in diameter in the silver preparations. These cells are very numerous and cover the outer portion of the entire nerve cord. They are less numerous in the anterior three or four segments. They have many collaterals which pass out into the general heart plexus.

For the investigation reported in this paper, the nerve cord was studied functionally and histologically as a whole and also in segments. These

¹ Dr. William Patton kindly loaned me some of his *Limulus* methylene blue preparations.

² I am indebted to Mr. R. S. Snider for making the preparations and counting the nerve cell.

segments were cut shorter and shorter until finally a very simple potential form was obtained. The recording apparatus consisted of a three-stage condensor coupled amplifier in association with a cathode ray oscillograph. Early in the work the coupling condensers used were of one-tenth microfarad capacity and the grid leaks one megohm. Because of the duration of the potentials obtained in the investigation the condensers were later changed to ones with two microfarads' capacity. This change permitted the recording of potentials lasting several hundred sigmas with very little

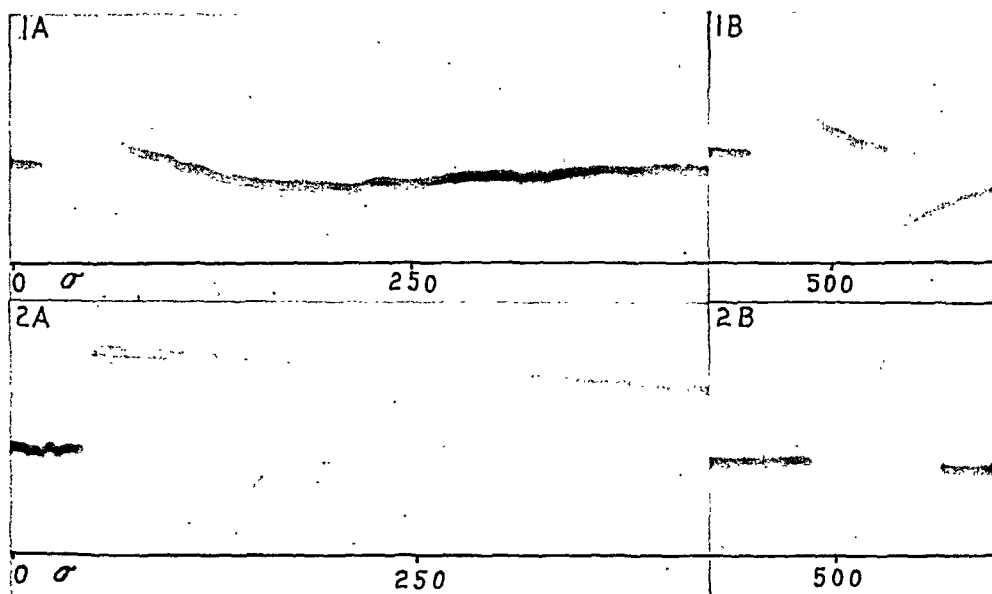


Fig. 2. Calibration of apparatus.

1A, record of output on closure of a constant current key, 0.1 microfarad coupling condensers, 1 megohm leaks, 250,000 amplification.

1B, record of output from input of constant current of relatively short duration, 0.1 microfarad coupling condensers.

2A, record of output on closure of a constant current key, 2 microfarad coupling condensers, 1 megohm leaks.

2B, record of output from input of a constant current of relatively short duration, 2 microfarad coupling condensers.

distortion. A calibration of the system is shown in figure 2. Potentials of the duration under investigation were considerably distorted by the small condensers and tended to show a diphasic artefact simulating a positive after potential. Records of the latter type were used only for certain phases of the investigation in which the actual form and duration of the potentials were not a first consideration. The recording electrodes were of the silver-silver chloride type and were so arranged as to permit a ready alteration of their position with reference to one another and to the preparation.

0 σ 200

2

0 σ

200

3

0 σ

200

Fig. 3. 1. Electroneurogram from portion of median cardiac nerve corresponding to second cardiomere; whole nerve cord intact because this part of structure is not spontaneously active. Note low potentials with no evidence of a sustained potential.

2. Electroneurogram from portion of median cardiac nerve of the same Limulus corresponding to the 3rd cardiomere; whole nerve cord intact. Note some increase in amplitude of potentials, still no evidence of a sustained potential.

3. Electroneurogram from portion of median cardiac nerve corresponding to the 5th cardiomere; whole nerve cord intact. Note evidence of a sustained potential. In this portion of a nerve cord are found large pacemaker cells. Multipolar cells are present in all portions of the nerve cord.

The complex potential record of the median cardiac nerve. The spontaneous potential record from the median cardiac nerve of *Limulus*, when recorded with two electrodes on live tissue, consists of periodic outbursts of irregular oscillations with positive and negative phases. These periodic discharges have the frequency of the heart beat. The form of the record varies in some important details in the different sections of the nerve cord. When the recording electrodes are placed on that part of the cord corresponding to the first three cardiomerer, the recorded potentials are lower than when similar leads are taken from the cardiomerer in the midportion of the nerve cord (fig. 3). Furthermore, there is never any evidence of the slow potentials which are nearly always present when the record is from the midportion of the nerve trunk. Very occasionally such slow potentials are not distinguishable in the record even from this portion of the nerve trunk. Even when present the slow potentials are not always visualized throughout their entire length. Their start is nevertheless sufficiently characteristic to always permit their identification (fig. 4).

In the light of evidence to be presented subsequently these findings assume significance. In that part of the nerve cord corresponding to the first three cardiomerer there are regularly none of the large unipolar ganglion cells which might therefore be held responsible for the slow potentials. In the midportion of the nerve cord there are collected all the giant unipolar ganglion cells and it is here that the slow potentials are developed. In those occasional instances where no slow potentials are recognizable in the records made from this portion of the nerve cord it is conceivable that they are developed simultaneously at both electrodes and consequently neutralize each other, only fiber potentials then being recorded.

Potential record from successively shorter stretches of nerve cord. Potentials recorded from two electrodes 10 to 14 mm. apart, each on live nerve from the mid-sections of the nerve cord show a slow potential with short spike potentials superimposed. When the tissue under one electrode is killed the superimposed potentials are thereby rendered more monophasic while the long lasting potential is unchanged (fig. 5). This is the result which would be expected if the brief potentials are conducted fiber potentials. Their complete monophasicity is not to be expected because it has been shown that non-myelinated fiber potentials show a large positive after potential (Bishop, 1934).

Potentials recorded from shorter and shorter stretches of nerve cord after killing under one electrode become simpler and simpler until in favorable preparations a single sustained negative deflection is obtained (figs. 5 and 6). On this single negative potential there are generally superimposed spike potentials of shorter duration and of the type characteristic of nerve fibers. Such spike potentials may be almost absent. The recorded magnitude of the large slow potentials varies with the freshness of

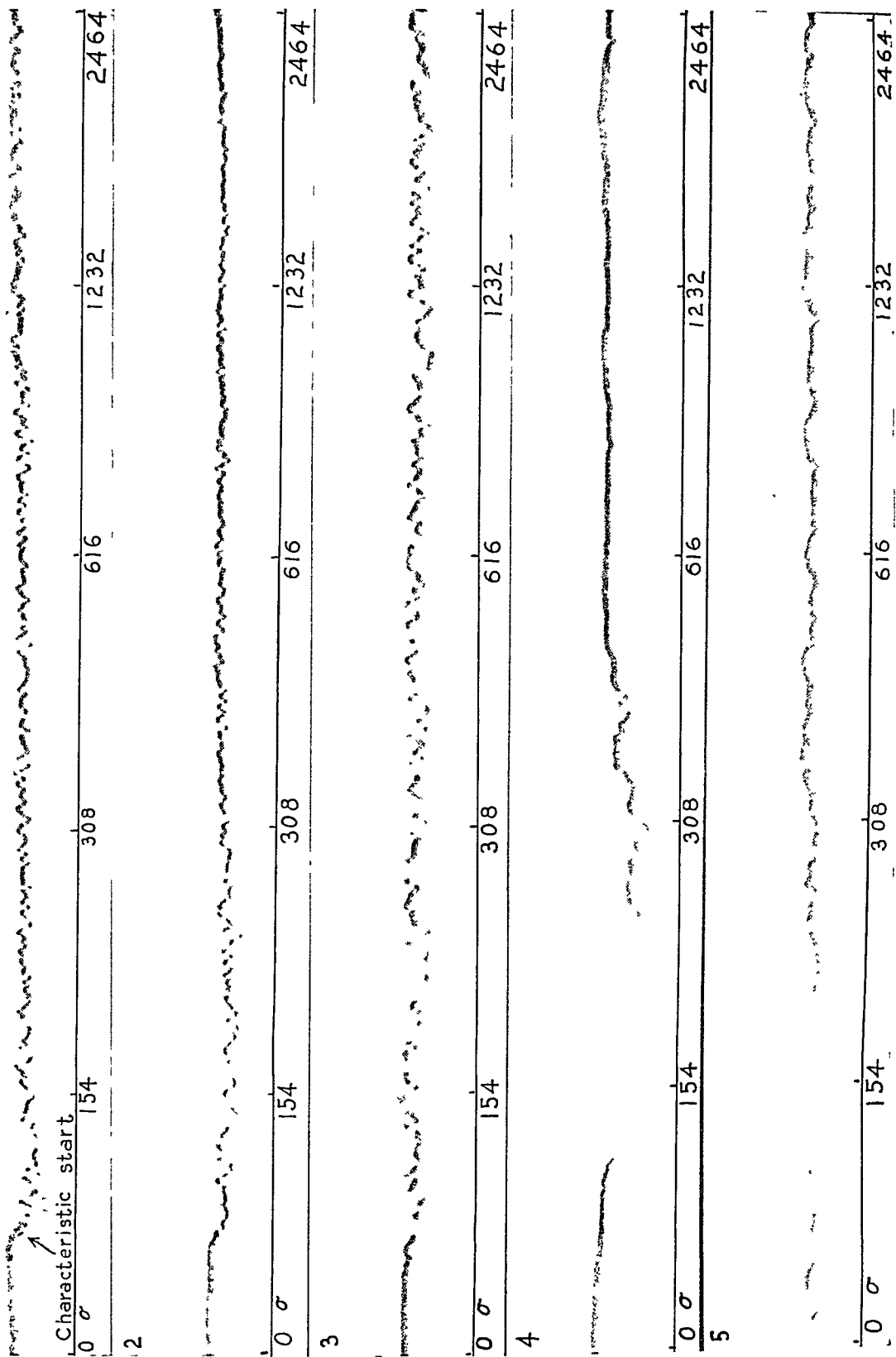


Fig. 4
692

the preparation from 0.1 to 0.25 millivolt. Their duration varies from 150 to 300 sigmas. Their duration under normal conditions varies with the frequency, being shorter when the rate of their occurrence is rapid. Their rising phase is approximately one-twentieth to one-tenth of the total duration. The duration of the slow potential remains unchanged by shortening the interelectrode distance.

Effect of altering position of the electrode with reference to a point of negative potential. Alteration of the position of an electrode to one or the other side of the nerve cord is without appreciable effect on the potential record. Shifting an electrode longitudinally 3 to 4 mm. with reference to a point of maximum negativity results in a lowering of the recorded potential without any appreciable change in its duration. There may be a reversal of the direction of the potentials when both electrodes are on live nerve. This might be determined by the relation of the cell to the electrodes. When one electrode is kept fixed on killed tissue, moving the other for some millimeters does not change the polarity. These experiments were made in the hope that some evidence might be secured of a directional polarity in the source of potential. The results seem to indicate that the entire cell surface becomes negative to the axon.

Correlation of functional and histological studies. The small portions of nerve cords, 3 to 6 mm. in length, from which smooth sustained potentials had been recorded, were fixed and stained according to the silver-on-slide method of Bodian. In serial sections, 10 to 30 microns in thickness, the nerve cells were identified as to type and counted. In two out of eight preparations a single large unipolar pacemaker cell was found in association with, in one case twenty-three of the multipolar cells, and in another thirty-seven. In the other preparations from two to ten giant unipolar cells were found in association with a somewhat larger number of multipolar cells. The two instances in which such single unipolar cells were found suggest the inference that here the long potential could have been the result of a single unipolar cell acting alone, because in regions where

Fig. 4. 1. Electroneurogram from portion of median cardiac nerve 8 mm. in length, 4 mm. interelectrode distance, rate of spontaneous discharge 24 per minute; 2 microfarad coupling condensers.

2. Electroneurogram after killing under grid electrode. Note marked simplification of record.

3. Electroneurogram 3 minutes after applying 1-2,000 strychnine sulphate. Note increased complexity and duration of record from activation by strychnine, rate 33 per minute.

4. Record after killing further under grid electrode, 3 mm. live nerve left. Rate 30 per minute. Note increased amplitude and duration of sustained potential as compared with 1.

5. Same preparation 4 minutes after application of 1-500 acetylcholine in sea water. Note lowering and shortening of sustained potential, rate 5 per minute.

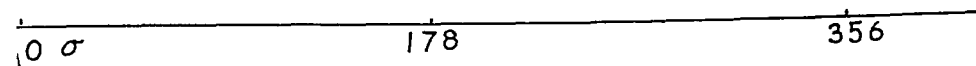
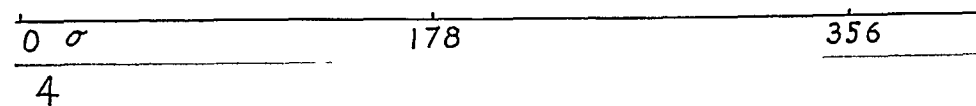
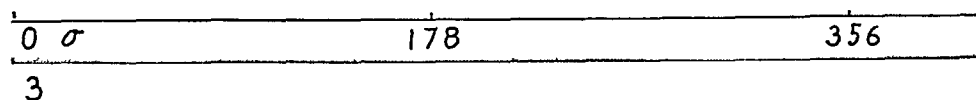
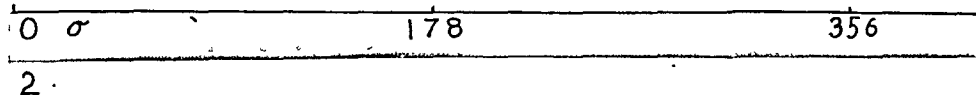


Fig. 5. 1. Electroneurogram from midportion of median cardiac nerve; total nerve length 14 mm., interelectrode distance 12 mm., both electrodes on live nerve. Coupling condensers 0.1 microfarad, 250,000 amplification. Note marked diphasicity of superimposed potentials.

2. Electroneurogram from same preparation after killing 4 mm. under grid electrode. Note simplification of record.

3. Electroneurogram after killing further toward ground electrode, 6 mm. live nerve now left. Note single sustained potential with a few superimposed short potentials.

4. Electroneurogram after further killing so that only 3 mm. of live nerve left under grid electrode.

multipolar cells exist alone they do not give rise to such long potentials on activation by other cells. They apparently also do not possess spontaneous activity. It is possible, in the preparations where more than one unipolar cell was found and where the sustained negative potential appeared simple, that not all the unipolar cells present were active. This is borne out by the effect of adding strychnine to certain of such preparations. Under such circumstances a simple potential may again become complex. The presumption is that strychnine excites some or all of the resting unipolar cells. In all the preparations crushing of the nerve could presumably have been carried farther and in many activity in a smaller number of cells might then have been shown to be associated with the sustained potential. The experiments were stopped to avoid destruction of the preparation for histological study. A more detailed study of the histology of the median nerve cord and of the nerve endings in the heart muscle is now being carried out.

Action of strychnine, acetylcholine, adrenalin and cocain on the complex activity potential and the sustained negative potential. When strychnine sulphate 1-2000 is diffusely applied to the median nerve cord the first effect is to increase the amplitude and frequency of the positive and negative phases of the potential complex, to increase its total duration and to increase the frequency of the spontaneous responses (fig. 4). The period of excitation is followed by a gradual depression so that in 15 to 30 minutes all spontaneous activity ceases. As the depression develops, it manifests itself by a slowing of the rate of total response, a lowering of the amplitude and frequency of the positive and negative phases of the potential complex and a decrease in its total duration.

These results are readily understood when the action of a similar concentration of strychnine sulphate on the sustained negative potential is studied on a short segment. The first effect is to increase the rate of its occurrence, to increase its amplitude and total duration (fig. 4). The frequency and sometimes the amplitude of the superimposed axon potentials are increased. The increased amplitude of the superimposed axon potentials when present is considered to be the result of a more perfect synchronization of the axon responses. Similar effects on the axon would be effected by the application of a galvanic current of increasing duration and strength.

Acetylcholine (1-500) in sea water applied to the nerve cord as a whole generally results in a slowing of the frequency of the total response, a lowering and shortening of the total potential complex with a lowering and slowing of the frequency of the positive and negative potentials. In a few preparations there has been observed for a few minutes an excitatory effect similar to that produced by strychnine. The depression from acetylcholine in the concentration employed is not rapid, 15 or more minutes are frequently required to depress to extinction all spontaneous activity.

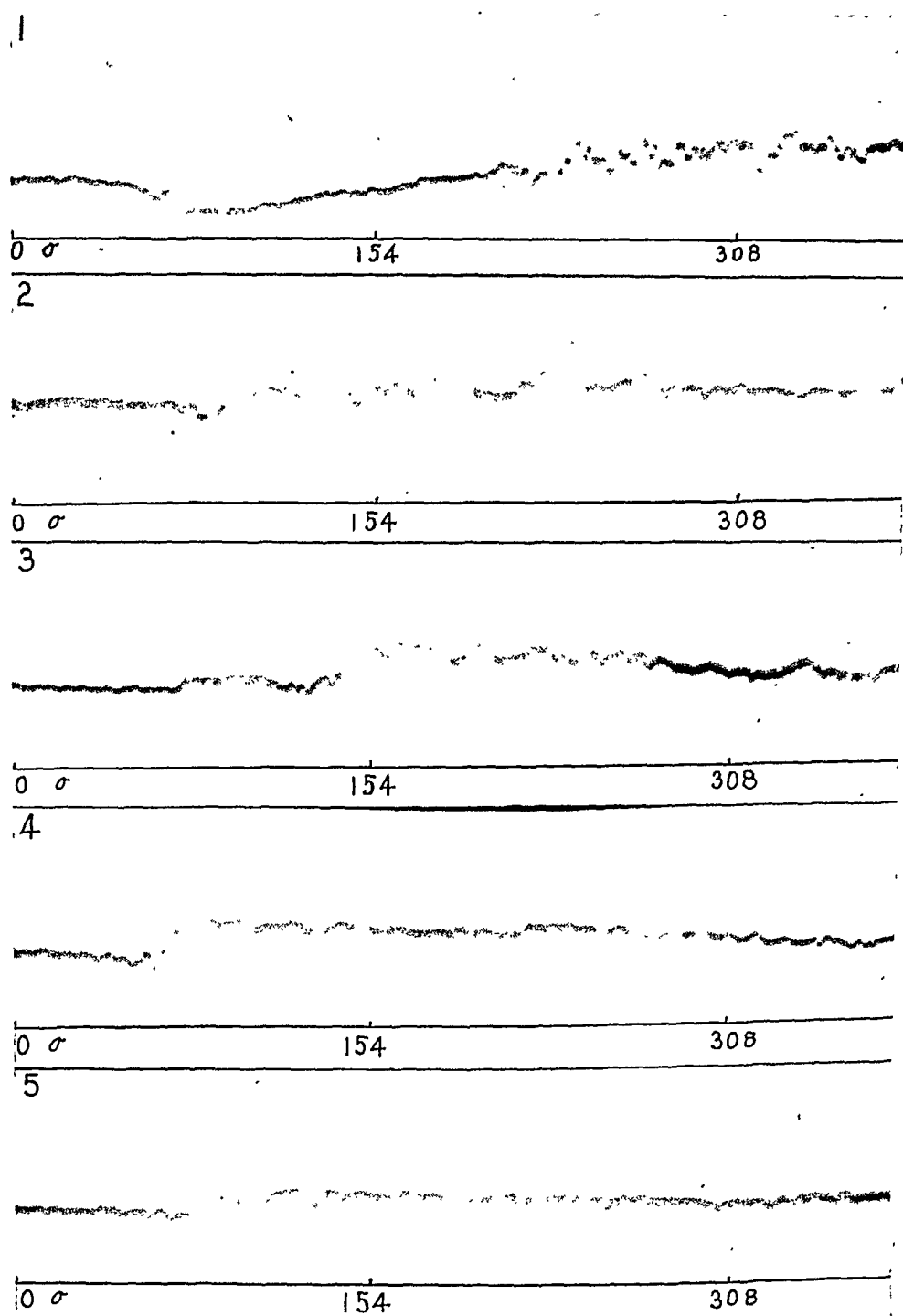


Fig. 6. 1. Electroneurogram from midportion of median cardiac nerve. Total nerve length 20 mm., interelectrode distance 11 mm., both electrodes on live nerve. Note unusual occurrence of a sustained potential with only a single superimposed

The effect of acetylcholine on the sustained negative potential is to decrease the frequency of its occurrence, to decrease its amplitude and to shorten its total duration (fig. 4). Superimposed axon potentials are decreased in frequency and often in amplitude. The depression of the rate of occurrence of the potential is relatively greater than is the depression of amplitude and duration of the potential. This is in contrast with the depressing action of cocaine when the depression in rate of occurrence parallels approximately the depression in amplitude and duration.

Cocain (1-100) in sea water first acts as an excitant similar to strychnine. With the concentration employed depression soon sets in with changes similar to those produced by acetylcholine except for the difference noted above. Adrenalin hydrochloride (1-100,000) acts as an excitant with effects on the potential records similar to those produced by cocaine as an excitant.

DISCUSSION. The problem of determining the source of the sustained potential is a difficult one. The evidence that the sustained potential becomes more and more simple on shortening the interelectrode distance and is most free of all superimposed oscillations when the live tissue is not over 3 to 4 mm. in length indicates that it is not the result of a summation of parallel fiber potentials. The amplitude and duration of such a summated wave would be greatest when the interelectrode distance was considerable and the number of active elements therefore greater. Moreover, it would not be expected that such a summated potential would show any appreciable degree of constancy of amplitude and of duration when the superimposed potentials as recorded are so irregular in form and in amplitude. It seems evident that the oscillations superimposed on the sustained potential are fiber potentials, both because of their form which corresponds to that of established fiber potentials recorded from the nerve and after stimulation and also because of the ability to make them monophasic or almost so on killing under one electrode. Failure to make the monophasicity complete is presumed to be due to the presence of positive after potentials in fibers of this type.

short potential until the base line is almost reached when other short potentials appear. Coupling condensers 2 microfarad, amplification 250,000.

2. Record with conditions same as in 1 after slight shifting of nerve on electrodes. Change of sign indicates the sustained potential is now recorded from the opposite electrode.

3. Electroneurogram after killing, 3 mm. of nerve under ground electrode. Note that superimposed short potentials tend to become monophasic.

4. Record after killing further toward grid electrode, 6 mm. live nerve left. Note further elimination of superimposed short potentials.

5. Record after further killing to leave 4 mm. of live nerve. Note some lowering of the sustained potential. This is not always the result on further killing. It is interpreted as an injury effect.

Assuming then that the sustained potential is a ganglion cell potential it is of interest to see to what extent the available evidence permits a designation of its source. There is first the evidence that the recorded potential from that part of the nerve cord corresponding to the anterior three cardiomereres when activated by impulses coming from the nerve cord more posteriorly does not show any of the sustained potentials. This anterior part of the nerve cord is not spontaneously active. Histologically it contains only cells of the multipolar type. It does not usually contain any of the large unipolar cells. The latter are concentrated in those cardiomere divisions which show a maximum degree of spontaneous activity. Our evidence shows that the presence of a single cell of this type, when associated with a few cells of the multipolar type, is adequate to produce a sustained potential. There is no correlation between the amplitude and duration of the sustained potential and the number of ganglion cells in the stretch of nerve giving rise to such a potential. The duration of the potential from a single cell with a few multipolar cells is as great as that recorded from preparations containing more than one unipolar cell and many more multipolar cells. While it is felt that the single cell itself is probably the source of such a sustained potential the evidence does not permit the statement as a demonstrated fact. Regardless of its source, the prolonged negative potential is associated with repetitive fiber responses. The stimulation effect on the fiber is regarded as a product of a potential gradient between axon and cell. The recovery period of the fiber is but a small fraction of the duration of the slow potential.

The spontaneously rhythmic ganglion cells of the heart cord appear to act in a manner similar to that of heart muscle which is itself rhythmic. Such rhythmic activity can be considered as a result of two processes, one the production of energy which, when it reaches a certain level, or threshold, induces spontaneous depolarization in the cell membrane, the other the depolarization of the cell membrane itself. Either of these processes might be altered by chemical or metabolic factors. Stimulation or inhibition of the energy building process might result in an increase or decrease in the rate of occurrence of ganglion cell depolarization. Alteration in the depolarization factor might result in changes in frequency and duration of its fiber responses. The latter, in turn, would determine the degree of the multipolar cell response. A nervous basis for the chronotropic and inotropic mechanisms of the heart of *Limulus* is thus suggested.

While the property of independent activity of an individual ganglion cell is not finally established by our results, they do demonstrate that the presence of a single cell of a certain type possibly in association with a small group of cells of a second type, in themselves incapable of spontaneous activity, may constitute a pacemaker mechanism. There is no reason

to believe that under the experimental conditions realized any excitant external to such a group was active in producing the cell activity. Such a cell can properly be designated as a pacemaker cell and it seems probable that it is the giant unipolar cell which serves such a function in cardiac nerve cord of *Limulus*. Such pacemaker cells are almost certainly present in other rhythmical centers such as the respiratory center. Adrian and Buytendyk's (1931) evidence of rhythmical activity in the gold fish brain with a rate corresponding to that of the animal's respiration adds support to this interpretation. Our findings in the *Limulus* heart cord correspond closely with those reported by Adrian (1931, loc. cit.) in his observations on potential changes in *Dytiscus marginalis*.

SUMMARY

Individual ganglion cells assumed to have pacemaker functions, or such cells in association with small groups of other cells in the median cardiac nerve of *Limulus polyphemus* show spontaneous activity when entirely separated from the body.

During such activity the single ganglion cell or such a cell in association with a small group of other cells, develops a sustained negative potential.

During this period of sustained negativity there is a repetitive nerve fiber response.

The sustained negative potential develops rather rapidly and then slowly subsides. There is no evidence of any appreciable positive after-potentials.

Activity in the fibers develops during the rising phase of the sustained potential and continues throughout the greater part of the period of negativity. The fiber response is most rapid during the height of the sustained potential and is slower as it subsides.

Strychnine, as an excitant, causes an increase in amplitude and duration of the sustained negative potential. The frequency of occurrence of the sustained negative potentials is increased as is the frequency of the fiber response during each period of sustained potential.

Acetylcholine as a depressant causes a slowing of the frequency of development of the sustained negativity and a lowering and shortening of the potential. There is a diminution in the frequency and duration of fiber response.

Adrenalin and cocain in concentrations causing increased excitability cause changes in the sustained negative potential similar to those produced by strychnine. As a depressant cocain decreases the amplitude of the sustained negative potential to a greater degree than does acetylcholine for a corresponding degree of slowing of the frequency of its occurrence.

REFERENCES

- ADRIAN, E. D. AND R. MATHEWS. *J. Physiol.* **65**: 273, 1928.
- ADRIAN, E. D. AND F. J. L. BUYTENDYK. *J. Physiol.* **71**: 121, 1931.
- ADRIAN, E. D. *J. Physiol.* **72**: 132, 1931.
- BISHOP, G. H. *J. Cell and Comp. Physiol.* **5**: 151, 1934.
J. Cell and Comp. Physiol. **8**, 1936.
- BODIAN, D. *Anat. Record* **65**: 89, 1936.
- ECCLES, J. C. *J. Physiol.* **85**: 464, 1935.
- ERLANGER, J., G. H. BISHOP AND H. S. GASSER. *This Journal* **78**: 537, 1926.
- FRÖHLICH, F. W. *Ztschr. f. Sinnes physiol.* **48**: 29, 1913.
- HARTLINE, H. K. AND C. H. GRAHAM. *J. Cell. and Comp. Physiol.* **1**: 277, 1932.
- HEINBECKER, P. *Proc. Soc. Exper. Biol. and Med.* **26**: 349, 1929.
This Journal **103**: 104, 1933.
- HEINBECKER, P., G. H. BISHOP AND J. O'LEARY. *Arch. Neurol. and Psychiat.* **35**:
1233, 1936.
- PATTEN, W. *The evolution of the vertebrates and their kin.* P. Blakeston's Son
and Co., Philadelphia, 1912.

OBSERVATIONS ON THE RESPONSE OF THE SPLEEN TO THE INTRAVENOUS INJECTION OF CERTAIN SECRETIN PREPARATIONS, ACETYL CHOLINE AND HISTAMINE

JOHN FERGUSON, A. C. IVY AND HARRY GREENGARD

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Illinois, and the University of Alberta, Edmonton, Canada

Received for publication August 3, 1936

We were interested in the effect of certain secretin preparations, or duodenal extracts, on the spleen because various reports in the literature indicate that a definite, but transient, erythrocytosis occurs after the injection of secretin preparations (1, 2, 3), and because a constriction of the spleen is believed to cause a temporary erythrocytosis (4, 5). Krzywanek (6), moreover, has suggested that the spleen plays a part in this phenomenon, basing his opinion on findings that the increase in erythrocytes per cubic millimeter after the injection of a secretin preparation was less in splenectomized than in normal dogs. However, he did not use a vasodilator-free secretin.

As we found that a certain secretin preparation (S_1 , vide infra) quite uniformly caused the spleen to constrict, we studied the effect of acetylcholine and histamine on the splenic volume, thinking that the response might possibly be due to minute traces of these substances. We were interested particularly in ascertaining the effect of acetylcholine on the spleen because Fredericq (7), studying the response of the excised organ of the dog to drugs and nerve stimulation, suggested that it may receive a parasympathetic innervation. We were unable to find a report in the literature regarding the effect of histamine on the splenic volume of the dog or on splenic strips.

METHODS. To determine directly the effect of certain secretin preparations, or duodenal extracts, on the volume of the spleen, we anesthetized a number of dogs (15–30 lbs. in weight), using sodium barbital or sodium pentobarbital, and ether. The spleen was enclosed in a plethysmograph and tracings were made before and after the intravenous injection of the substances studied. A blood pressure record was made simultaneously. The preparations used are designated S_1 , Aniline-filtrate, and Aniline-precipitate by Ivy et al., and are described in their publications (8, 9, 10).

S_1 is a preparation which contains both secretin (active in 0.25–0.5 mgm.

doses) and cholecystokinin (active in 2.5–3 mgm. doses). The aniline-filtrate preparation contains chiefly secretin and is active in 0.05–0.025

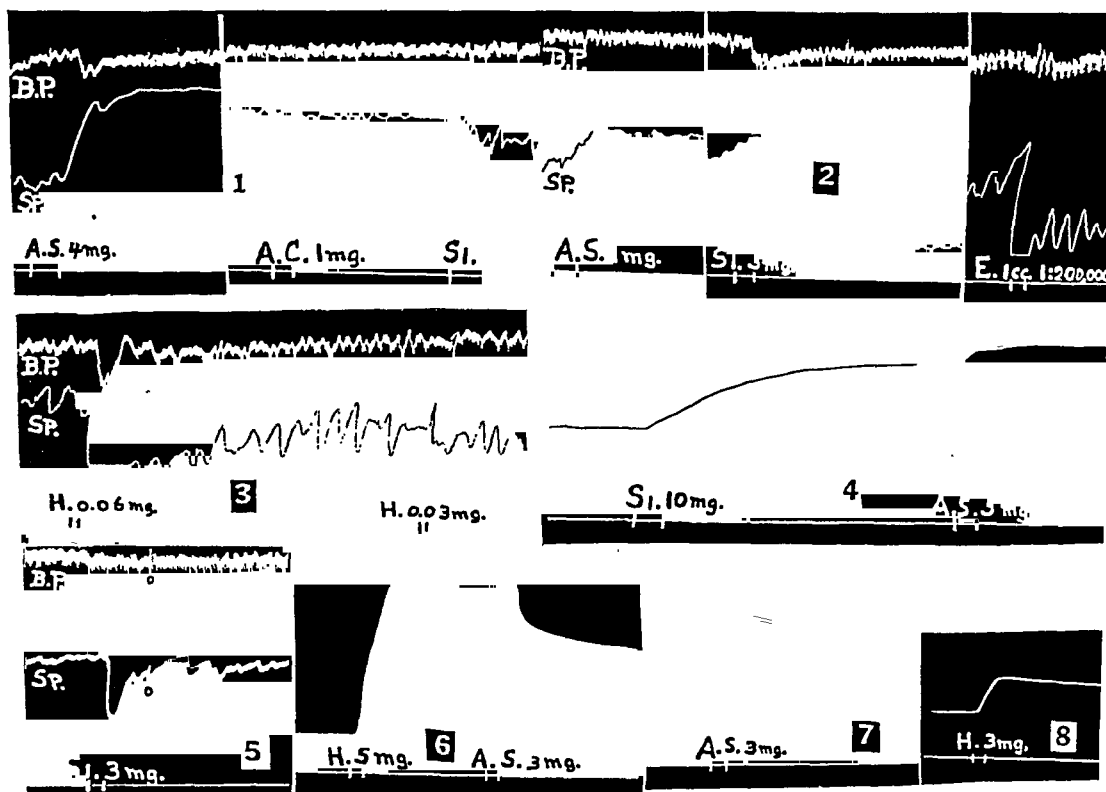


Fig. 1. *B. P.*, blood pressure; *Sp.*, spleen volume; *Up*, dilatation; *Down*, constriction; *A. S.*, atropine sulphate, *A. C.*, acetyl choline; *E.*, epinephrine; *H.*, histamine.

Tracing 1 shows dilatation after atropine and the failure of *A. C.* to work 10 minutes later; *S₁*, however, caused a constriction 5 minutes after the *A. C.*

Tracing 2 shows dilatation after atropine followed by a constriction of the spleen 5 minutes later from *S₁* and then a few minutes later epinephrine 1 cc. 1:200,000 caused an analogous constriction except that it was of shorter duration.

Tracing 3 shows constriction of the spleen from 0.06 mgm. of histamine together with a drop in blood pressure and the ineffectiveness of 0.03 mgm. to lower the blood pressure or to constrict the spleen.

Tracing 4 shows the contraction of a splenic strip brought about by 10 mgm. of *S₁* and the ineffectiveness of atropine to inhibit the action; in fact, atropine appeared to cause contraction.

Tracing 5 shows a typical response of the spleen to 3 mgm. of *S₁*.

Tracing 6 shows a contraction of a splenic strip from 5 mgm. of histamine, and a relaxation induced by 3 mgm. of atropine.

Tracing 7 shows a typical relaxation of a splenic strip from atropine.

Tracing 8 shows a typical contraction of a splenic strip from 3 mgm. of histamine.

mgm. doses. It stimulates the gall bladder slightly in 1 mgm. doses. The aniline-precipitate preparation contains only traces of secretin, but contains some active cholecystokinin (active in 5 mgm. doses).

RESULTS. *S₁ preparation of "secretin."* Twenty out of thirty dogs manifested a definite constriction of the spleen after the injection of 5 mgm. of *S₁* preparation. The minimum effective dose was 2 mgm., or four threshold doses of secretin. A constriction of the spleen was quite uniformly obtained with successive injections in responsive animals; in twenty reactive animals, we recorded forty-five constrictor responses in sixty-three trials. A dilator response was not observed. The result of a typical constriction of the spleen is shown in figure 1, tr. 5. In volume the constriction obtained using 3 to 5 mgm. of *S₁*, generally amounted to from 2 to 3 cc., occasionally more. The degree of constriction obtained was comparable to that observed after the injection of 1 cc. of a 1:200,000 solution of epinephrine. The constriction was not prevented by atropine sulphate (4 mgm.); but occasionally during a post-injection-atropine period of about ten minutes the constriction appeared to be reduced, after which period the usual constrictor response was obtained. From 1 to 4 mgm. of atropine sulphate were used (fig. 1, tr. 1 and 2).

The aniline-filtrate preparation of "secretin." This preparation was used as we believe it to be almost pure secretin. Although this preparation was injected eighteen times into eight dogs in doses as large as 1 mgm. (20 plus doses of secretin), a definite constriction of the spleen did not occur. In five tests a very slight decrease in volume resulted; in five others an increase in the rhythmical changes in volume occurred; and in eight trials no change or a very slight change in volume occurred. These results showed that the substance in the *S₁* preparation which caused the constriction of the spleen was either in the aniline-precipitate or had been destroyed by the chemical treatment. These results, also, showed that the substance in *S₁* causing constriction of the spleen is not secretin.

The aniline-precipitate preparation. The aniline precipitate was injected twenty-four times into nine dogs in doses as large as 10 mgm. In no instance was a definite change in the volume of the spleen observed. In two cases, one in which a dose of 10 mgm. and the other in which 5 mgm. were injected, a slight constriction occurred each of which could be interpreted as a spontaneous variation.

The failure of the aniline filtrate and precipitate to cause a definite constriction shows that the aniline method of concentrating secretin inactivates the substance in duodenal extract which causes constriction of the spleen.

Not being able to concentrate the splenic constricting substance in *S₁*, we decided to ascertain if the constriction might be due to traces of acetyl choline or histamine. These substances, if in the *S₁* preparation, must be present only in minute traces because the systemic blood pressure was never more than slightly influenced by the injection of *S₁*.

Acetyl choline. One milligram of acetyl choline caused a constriction of the spleen. The amount of constriction compared favorably to that

obtained with from 3 to 5 mgm. of S_1 . The constriction persisted considerably longer than the fall in blood pressure which indicated a true contraction of the spleen. This interpretation is supported by the observation that in two instances during an atropine recovery period the spleen showed constriction on the injection of acetyl choline without a change in blood pressure. We always obtained a constriction of the spleen with acetyl choline, and are at a loss to explain the variable responses observed by Hunt (11).

Atropine sulphate. Atropine, contrary to the results of Krafka, McCrea and Vogt (12) on the excised spleen of the hog and calf, in 1 mgm. doses caused a slight, and in 4 mgm. doses a definite dilatation of the spleen (fig. 1, tr. 1 and 2). Atropine sulphate in 1 mgm. doses abolished the constrictor action of acetyl choline for about ten minutes; in 4 mgm. doses atropine abolished the constrictor action for about fifty minutes. However, in one dog a typical constriction was obtained 20 minutes after 4 mgm. of atropine and before the typical effect of acetyl choline on the blood pressure had returned.

Histamine. Histamine in doses which definitely reduced the blood pressure caused a contraction of the spleen (fig. 1, tr. 3). It was found that 0.0625 mgm. (10–15 kilo dogs), which caused a fall in blood pressure of 24 mm. of Hg, resulted in a constriction which was approximately equivalent to that caused by 3 to 5 mgm. of S_1 , which outlasted the period in the fall in blood pressure by from 5 to 10 minutes. A dose which would cause only a 10 to 15 mm. fall in pressure would cause a definite constriction lasting 5 or 10 minutes. A dose, such as 0.0321 mgm. (15–18 kilo dog), which produced no fall in blood pressure, caused little or no constriction. Thus it follows that the constriction of the spleen after histamine administration is related directly to the fall in blood pressure.

Use of splenic strips. In order to confirm our results on the intact animal when using the various substances mentioned above, we determined the effect of these drugs on strips of fresh spleen of the dog suspended in a bath.

Methods. The strips were approximately 5 cm. in length and included the more muscular part of the spleen, that is, the inner part of the capsule. The bath in which the strips were immersed was of a composition recommended by Sollmann and Rademaekers (NaCl 0.9 per cent; KCl 0.042 per cent; $CaCl_2$ 0.012 per cent; $NaHCO_3$ 0.03 per cent; glucose 0.1 per cent). The pH of such a solution is 7.8. It was oxygenated by a stream of air, which was washed with a 0.1 per cent solution of sodium bicarbonate. The temperature of the bath was held at 37.5°C. The volume of the bath was approximately 100 cc. In many instances it was necessary to use a fresh strip for each test as a spontaneous return to normal after a strong contraction is so long delayed that the further use of the strip is impracticable.

Results. Acetyl choline. Fredericq (7) and Vairiel (13) reported that acetyl choline will cause a contraction of a splenic strip and that epinephrine will induce an additional contraction. Working on splenic strips in a bath as indicated above, we obtained a contraction using acetyl choline and an additional contraction with epinephrine. After adding 1 mgm. of atropine sulphate to the bath, 3 mgm. of acetyl choline were ineffective.

Atropine sulphate. Atropine sulphate when added to the bath caused a prolonged relaxation of the splenic strip. Doses of 3 to 5 mgm. appeared to give maximal effects. Typical results are shown in figure 1, tr. 6 and 7.

Histamine. The results from histamine were somewhat variable. In the majority of cases the strip contracted (fig. 1, tr. 6 and 8), but in certain instances a slight relaxation occurred.

S₁ preparation of secretin. S₁ in dose of 10 to 20 mgm. gave a marked contraction of the splenic strip (fig. 1, tr. 4). From the tracing it is clear that atropine does not counteract the effect of S₁; in fact, atropine either causes a contraction or sensitizes the strip to S₁. This odd phenomenon was not studied further because the supply of S₁ was limited.

DISCUSSION. Studying the action of histamine on the spleen of the cat, Dale and Laidlaw (14) found that it causes a constriction which did not last as long as the fall in blood pressure. They thought that histamine caused an active contraction of the musculature of the spleen and that this was a more important factor than the fall in blood pressure. However, they did not use doses of histamine which have no effect on systemic blood pressure, as we did, and our results show that to obtain constriction of the spleen with histamine a dose which causes some fall in blood pressure must be employed. Hence, the constriction of the spleen *in situ* caused by histamine is either a response caused by a fall in blood pressure or the excitatory dose of the chemical for the splenic musculature is identical with the vasodepressor dose. Relatively large doses of histamine caused, as a rule, a contraction of splenic strips, although the effect was variable. It is possible that *in situ* histamine reduces the volume of the spleen by causing its musculature to contract, but in ordinary doses this seems unlikely. Regardless of the mechanism by which histamine causes constriction of the spleen, it is evident from our results that the constrictor substance in the S₁ preparation is not histamine, which was the point of chief interest to us.

Our observations on the effect of acetyl choline and atropine on the volume of the spleen and on splenic strips corroborate the observations of Fredericq (7) on the effect of these drugs on the isolated spleen. Our *in vivo* and Fredericq's *in vitro* results, which we confirmed, show that the splenic musculature is responsive to parasympathomimetic as well as sympathomimetic drugs. The fact that we occasionally observed recovery of the spleen from the atropine block of acetyl choline before the blood

pressure effect returned indicates that the splenic musculature is somewhat analogous to that of the intestine in being refractory to atropine.

It is clear that neither histamine nor acetyl acholine is the spleno-constrictor substance present in our S_1 preparation for the following reasons: *a*, the injection of S_1 frequently causes constriction without the slightest change in blood pressure and when a change in blood pressure does occur it is so slight as not to explain the unproportionate or marked constriction of the spleen; *b*, the constriction of the spleen caused by histamine *in vivo* is directly related to the change in systemic blood pressure because a constriction of the spleen was never obtained by a dose of histamine too small to cause a change in blood pressure; *c*, the constrictor effect of acetyl choline is counteracted by atropine for from ten to fifty minutes, while the constrictor action of S_1 is counteracted at no time by atropine. The same is true for splenic strips. We have no explanation to offer for the fact that some dogs appear to be refractory to the S_1 preparation, whereas they are not refractory to acetyl choline, histamine, or epinephrine. The musculature of the duodenum, though quite uniformly caused to contract by this preparation (S_1) is also refractory in some dogs (15).

Since a preparation of secretin can be made which in relatively large doses has no definite constrictor action on the spleen, secretin is not the spleno-constrictor substance in duodenal extracts. We suspect that the spleno-constrictor substance in our S_1 preparation from duodenal mucosa is the same substance observed frequently to cause contraction of the duodenum and intestine in the cholecystokinin preparation used by Sandblom, Voegtlin, and Ivy (15) and practically absent from the cholecystokinin preparation used by Lueth, Ivy and Kloster (16). For this reason and because our aniline precipitate contains cholecystokinin, we believe, at present, that the spleno-constrictor substance is not cholecystokinin. Yet, a variation in the threshold irritability of the gall bladder, duodenal and splenic musculature may explain our results. We suspect, however, that an excitant of the intestinal and splenic musculature other than cholecystokinin, acetyl choline and histamine will be isolated at some future date from the duodenal mucosa (17). To date we have had no success in preparing such a motor excitant free from both secretin and cholecystokinin.

CONCLUSIONS

1. A spleno-constrictor substance is present in certain extracts of the duodenal mucosa.
2. The spleno-constrictor substance is not acetyl choline, histamine, or secretin and probably is not cholecystokinin.
3. We have been unable to concentrate by fractionation the spleno-constrictor substance, but point out in the discussion that it is probably

identical with the substance in the duodenal extract which augments intestinal motility as referred to by Sandblom, Voegtlin and Ivy.

4. Acetyl choline causes contraction of the spleen *in vivo* and contraction of strips of the spleen. Its action is antagonized by atropine.

5. Certain duodenal extracts cause contraction of splenic strips which is not antagonized by atropine.

6. Histamine causes contraction of the spleen *in vivo* in doses that affect blood pressure and relatively large doses of histamine are required to cause contraction of splenic strips.

7. Atropine causes relaxation of the spleen *in vivo* and relaxation of splenic strips.

REFERENCES

- (1) DOWNS, A. W. AND N. B. EDDY. This Journal 43: 415, 1917.
- (2) FUJIMOTO, B. This Journal 47: 342, 1918.
- (3) KING, J. F. Arch. Int. Med. 42: 763, 1928.
- (4) BORYSIEWICZ, A. Compt. rend. Soc. de Biol. 99: 931, 1928.
- (5) BARCROFT, J. AND L. T. POOLE. J. Physiol. 64: 1, 1927-28.
- (6) KRZYWANIEK, F. W. Pflüger's Arch. 222: 435, 1929.
- (7) FREDERICQ, H. Compt. rend. Soc. de Biol. 101: 1164, 1929.
- (8) IVY, A. C., G. KLOSTER, H. C. LUETH AND G. E. DREWYER. This Journal 91: 336, 1929.
- (9) IVY, A. C., G. KLOSTER, G. E. DREWYER AND H. C. LUETH. This Journal 95: 35, 1930.
- (10) VOEGTLIN, W. L., H. GREENGARD AND A. C. IVY. This Journal 110: 198, 1934.
- (11) HUNT, R. This Journal 45: 197, 1918.
- (12) KRAFKA, J., JR., F. D. MCCREA AND E. VOGT. J. Physiol. 68: 292, 1929-30.
- (13) VAIREL, J. J. de Physiol. et de Path. Gen. 31: 42, 1933.
- (14) DALE, H. H. AND P. P. LAIDLAW. J. Physiol. 41: 318, 1910.
- (15) SANDBLOM, P., W. L. VOEGTLIN AND A. C. IVY. This Journal 113: 175, 1935.
- (16) LUETH, H. C., A. C. IVY AND G. KLOSTER. This Journal 91: 329, 1929.
- (17) IVY, A. C. J. A. M. A. 105: 506, 1935.

STUDY OF DEPTH TEMPERATURES IN ARTIFICIAL FEVERS AND COOLING AIR CHAMBERS WITH ESPECIAL REFERENCE TO COOLING EFFECT OF THE CIRCULATING BLOOD

JOHN J. SAMPSON

From the Mount Zion Hospital and the Department of Medicine, University of California, San Francisco

Received for publication July 15, 1936

Studies of deep internal temperature in man have been made since the time of Becquerel and Breschet (1), who in 1835 first used electrical thermocouples for this purpose. Recent investigations in this field have been made by Lefevre (2), Zondek (3), Bazett and McGlone (4), Foged (5) and Wright and Johnson (6). Of early animal experimentation, Claude Bernard (7), Liebig (8) and Colin (9) did the most extensive work demonstrating the heating of the blood by the somatic muscles, the liver and the intestines, and cooling of the blood by surface exposure.

It is recognized that heat gradients exist in man in zones from the skin surface to various depths and that these gradients vary under circumstances of formation or loss of heat throughout the body and may vary in different portions of the same individual's body at the same time. As suggested by Bazett (10) and Deighton (11) but, to my knowledge not yet proved, the gradient from the skin to deeper tissues may be reversed on recovery from cold or onset of fever, and Bazett and McGlone (4) have shown that the deep skin layer may be cooler than the superficial one.

It is the purpose of this paper 1, to show that such gradient reversals are exhibited in artificial fever and occasionally at normal temperatures; 2, to confirm the observations that cubital venous blood is usually cooler than most tissues, and that gradient changes are sudden from skin to the generally cool subcutaneous tissue and warm striated muscle; and 3, to demonstrate that, whereas, blood may serve to heat tissues on returning from a heated skin or active muscles and tissues, it may often be an important cooling agent for body tissues. Especially when skin cooling is excluded, is it apparent that much heat is lost from the blood in the respiratory tract.

TECHNIQUE. The apparatus used for studying such temperatures in the human body was a copper-cupron (a copper nickel alloy) thermocouple junction imbedded in a bakelite collar in the tip of a no. 22 gauge rustless steel needle, 10 cm. in length, with the customary circuit of galvanometer

and thermocouple connection in a water bath of known temperature. The apparatus was calibrated and thermocouple readings checked by standardized clinical thermometers in the oral and rectal readings. The readings could be made in less than five seconds and the needle apparatus showed no demonstrable tendency to conduct heat away from the thermocouple junction in this period. In taking intravenous temperature the thermocouple needle was inserted into a no. 18 gauge steel needle that was placed in the vein in such a manner as to project about 2 mm. beyond the tip of the latter needle.¹

The entire arm, except at the point of puncture, was kept warmly but not tightly covered when intravenous blood temperature readings were made in the cubital vein, and was not removed from the blankets when observing temperatures in artificial fever cases. The normal men were put in warm beds for $\frac{3}{4}$ to 1 hour before temperature readings were made. Four adult men with normal temperatures were observed, seven with fever produced by swathing in blankets and rubber sheets by the Epstein and Cohen technique (12), two men with fever produced by both electric light cabinet and blanket swathing and one by intravenous administration of typhoid-vaccine. The cooling effect of inclosing the head and neck in a tent in which supercooled air was circulated was studied in two men with normal temperatures and one with "blanket pack" fever, and the cooling influence of cold sponge baths was studied on two of the artificial fever cases.

RESULTS. *General normal temperature gradients.* Typical observations are illustrated in table 1. The relation of the various normal temperatures agreed in general with the findings of Wright and Johnson (6), Bazett and McGlone (4) and others. In one man (Mr. V., table 1) whose layer of

¹ I am indebted to W. W. Salisbury for the construction and testing of the thermocouple needles used. With 10°C. difference in the temperature between the measured point and the room, his estimation of heat loss in the no. 42 gauge copper wire of the thermocouple was 4.6×10^{-6} calories per second and of the no. 42 gauge cupron wire was 4.5×10^{-7} calories per second. Assuming the heat conductivity of water as 0.0014 and that blood approximates this, a maximum error of 0.065°C. may occur but much less than that if the blood is in motion. Assuming that human fat approaches the heat conductivity of castor oil, namely, 0.000425, the maximum error in the subcutaneous tissue reading could be 0.25°C. The conductivity of muscle probably lies between the above two estimates.

The bakelite sheath is an excellent heat insulator and with at least 1.5 cm. of the needle buried in tissue, little significant error can be attributed to heat loss from these sources.

The galvanometer reading actually became stationary within one second in all observations except those of the skin, in which the above errors may have been present, and in the intravenous readings in certain infrequent instances when the skin was temporarily uncovered to place the thermocouple and then recovered to attain its previous temperature.

superficial fat was unusually thin, temperatures were much lower in all the tissues of the exposed arm than in the covered thigh. Mark and later Wieland (cit. Deighton, 11) have previously explained such findings as evidence of the insulating property of fat. Intramuscular temperature exceeded the rectal temperature in only one of seven cases but exceeded or equalled the oral temperature in three cases. The intravenous blood temperature was lower than all but the surface skin temperature in five out of seven cases, excluding the deltoid region temperature readings in Mr. V. (table 1). In the other two cases only the temperature of the subcutaneous tissue as well as the skin was lower than the intravenous readings.

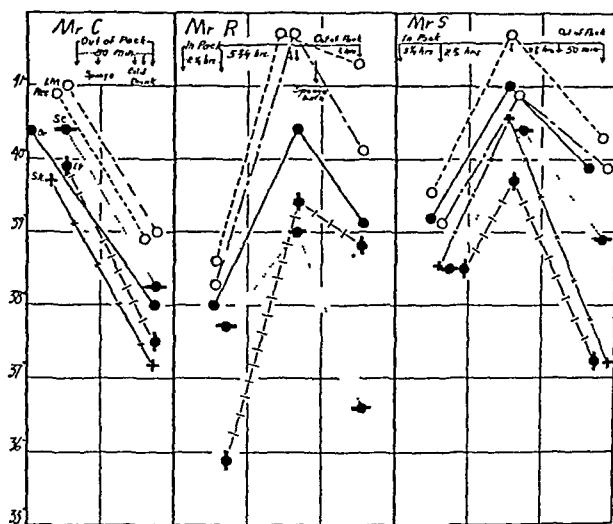


Fig. 1. Temperature relations in cooling by surface sponging in artificial fever cases. *I-M*, *S-C*, *I-C*, *Sk*, or *Cut* refer respectively to intramuscular, subcutaneous, intracutaneous, and surface skin temperature readings of the same thigh region and "*I-V*" to intravenous blood (median basilic vein). Temperature readings all by the thermocouple needle method. *Or* and *Rec* indicate oral temperature and rectal temperature respectively as taken by clinical mercury thermometers. Such readings were frequently checked against thermocouple determinations.

Three out of the seven cases with normal temperatures exhibited skin surface temperatures equalling or exceeding those of the subcutaneous tissue (table 1—case of Mr. A.—quadriceps-femoris region; Mr. V.—deltoid region) (fig. 1—Mr. C.—buttocks region). Insofar as these cases did not represent general temperature changes when the observations were made, it is difficult to account for this phenomenon otherwise than by loss of heat from the surface elsewhere than in the region studied, or by a general cooling effect of arterial blood. The latter was assumed to be the sole operative mechanism in the "blanket pack" fever cases.

Temperature gradients with artificial fever. This reversal of skin-subcutaneous tissue gradient was the common finding in cases of "blanket pack" fever production (four out of six observations) and was sometimes associated with a wider separation between temperatures of the venous blood and the other tissues; the former rising more slowly than the rectal, oral or intramuscular temperatures (fig. 2). Herein is demonstrated an instance in which the blood assumes the chief cooling function of the body, since with no evaporation possible, little heat can be lost from the skin. The temperature in the deep layers of the blankets generally approached the oral temperature and exceeded the skin temperature.

TABLE 1
Comparative temperatures of individuals without fever (Centigrade)

	RECTAL	ORAL	INTRAMUSCULAR		SUBCUTANEOUS		SKIN SURFACE		INTRACUTANEOUS		INTRAVENOUS
			Deltoid	Thigh	Arm	Thigh	Arm	Thigh	Arm	Thigh	
Mr. A., well-nourished.....	37.7	37	36.4	36.45	34.9	34.85	34.3	34.9	31.7	33.8	35.6
Mr. V., mal-nourished.....	37.4	36.6	35.75	36.9	32.9	36.3	33.6	35.5	31.3	34.3	34.4
							Skin neck				
Mr. R.....	37.1	36.6		37.7		36	35	35	32	32.5	35
			RECTAL		ORAL						
			Superficial	Deep							
Mr. D., hepatic cirrhosis.....			37.5	36.8	36.8		Abdominal wall Ascitic fluid Intra-peritoneal space			37.4	

Another good example of this reversal of the gradient of the skin and subcutaneous tissue in which the rôle of the blood is more clearly evident, occurs in case 3, Mr. V., figure 2. In this case the patient was lightly clothed and exposed to the hot air in the electric cabinet for a sufficiently prolonged period to produce fever. The skin still remained cooler than the other tissues, as evaporation could still take place. The blood, however, was carried away from the surface appreciably warmed, its temperature exceeding even that of the subcutaneous tissues. The normal gradient was still maintained with this exception. On removing the cabinet and wrapping the patient in blankets the reversal of the gradient occurred. The skin was no longer an effective means for heat loss and became warmer

than the subcutaneous tissue. Increased metabolism of the skin itself may partially account for its high temperature under such circumstances. The blood temperature actually fell since it then became an agent of cooling from within instead of heating from without.

Temperatures recorded deep in the skin were always the lowest observed in any patient but were slow to come to equilibrium. Such low readings may be due to shutting off adjacent blood supply by pressure of the needle in the firm tissue layers and may not, therefore, represent a true state. Deep skin temperatures, low relative to that in other tissues, likewise have

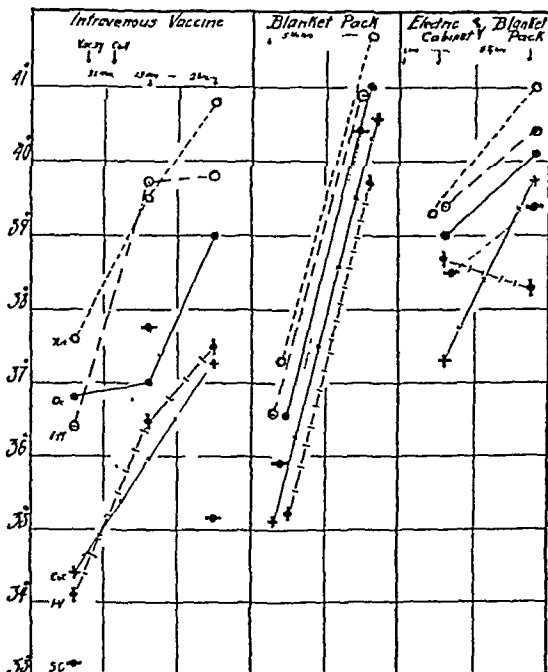


Fig. 2

Fig. 2. Temperature relations in artificial fevers. Symbols as in figure 1.

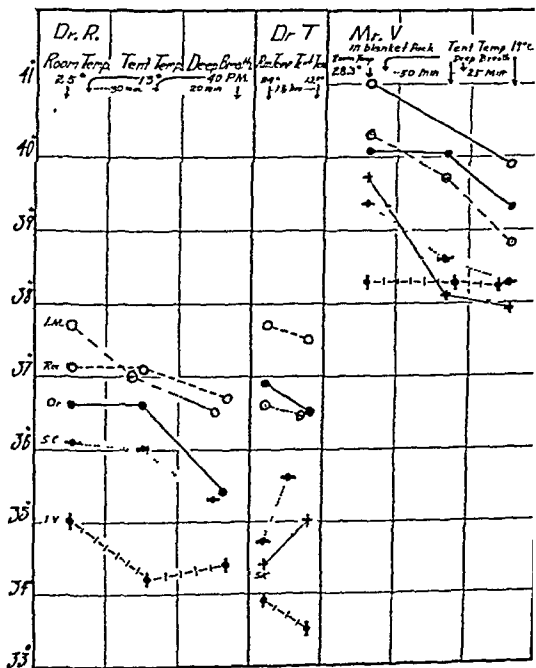


Fig. 3

Fig. 3. Temperature relations in artificial cooling by iced air. Symbols as in figure 1.

been reported by Bazett and McGlone (4). Intramuscular temperatures generally rose somewhat more rapidly in the "blanket pack" fever cases than oral or rectal, exceeding the latter temperature at the height of the fever in four out of six cases. (Fig. 1—cases 1 and 2.) The prompt fall of intramuscular temperatures upon release from the "blanket pack," equaling the rate of subcutaneous tissue temperature drop, suggests that the mechanism of fever production was probably purely one of retention of heat and not excess formation in the muscles or other active tissues. If such excess heat formation existed, some lag of the temperature fall curve might be expected.

The behavior of intramuscular temperature in the vaccine fever case (case 1, fig. 2), is interesting because it rises rapidly with the chill as expected, but fails to continue upward with the rising oral and rectal readings. One may deduct that surface heat loss, as evidenced by the falling subcutaneous temperature, equals the heat formation in the muscle which is obviously less in degree than with the contractions during the chill. It is then assumed that excess heat formation proceeds at a more even rate in the abdominal organs and because of their depth the generated heat is better retained than in the superficial muscles. The constant rise of venous blood temperature suggests that the elevated general body temperature cannot be easily explained in the presence of a loss in subcutaneous tissue heat except as previously discussed.

Temperature gradients with general body cooling. Certain mechanisms of heat loss are illustrated in two cases with surface sponging at the peak of the fever (fig. 1). All temperatures fell uniformly in the first case but the intravenous temperature, proportionately to other temperatures, fell more slowly in the second. This behavior resembles the level state of the intravenous temperature in figure 3—Mr. V.—when other tissues were cooling, and illustrates how the blood may act as a temperature stabilizer. Its high specific heat tends to maintain a gradient in a warmer or cooler region, permitting the removal or supply of heat without the blood rapidly approaching the temperature of its environment.

It has been previously illustrated how blood serves as a cooling agent when the skin is eliminated as the chief source of heat loss. The respiratory rates of these patients with artificial fever are commonly over 25 per minute which acceleration would tend to increase the heat dissipating function of the respiratory tract. If this mechanism alone were adequate it is apparent that little fever would be produced. An index of this adequacy is the slow rise of the general body temperature (oral and rectal) and its maintenance at a maximum of over 40°C. orally for 3 to 5 hours with little further rise in spite of continuance of the pack. Thus one may suggest that in man the respiratory tract has great potential means for heat loss, at least under special circumstances.

Tigerstedt (13) estimated that 5 per cent of all the heat loss normally occurs in warming air and ingested fluids and food and 10 per cent in release of CO₂ in the lungs and saturating inspired air with water (to 95± per cent). This estimate must be erroneously low under the circumstances of unusually cold or dry air, rapid or deep respiration, or fever. Barach (14) and others have described the fall in temperature in pneumonia patients placed in cooled oxygen tents. Exclusive of the rapid respiration rate of such patients, the continued inspiration of cool, dried air in such apparatus operates to drop the general body temperature. This is illustrated by the following observations.

Figure 3 illustrates two series of temperature observations of normal individuals (Drs. R. and T.) before and after inspiring iced air in a bed tent. At first their respiratory rates were 18 per minute and later 32 to 40 per minute. The fall of all tissue temperatures in the case of Dr. R. was definite, especially after rapid deep breathing. As previously stated, the tent was placed over the head and neck and the remainder of the body remained at room temperature. The ordinary reaction to local cold (Bazett) is a general contraction of skin capillaries which tends to retain heat and not drop deep tissue temperature. It may be assumed that the blood was overcooled leaving the lungs, and served as a general cooling agent to all body tissues. This is strongly suggested in the observations on Dr. T. (fig. 3), since the skin and subcutaneous tissue actually were warmer at the end of the experiment than at the beginning. This was probably caused by the tissues approaching equilibrium with the higher room and bed temperatures on recent return of the individual from exposure to the lower outdoor temperature.

The temperature drop in various tissues in the artificial fever case in which a similar iced air tent was used was striking. However, the stability of the venous blood temperature fails to suggest unusual arterial blood cooling. It thus resembles the observations made on the cooling of fever patients by surface sponging and recalls the comment made on the high specific heat of blood. Some of the heat loss in these iced air tent experiments must take place from the face as well as from the respiratory tract and it is admitted that this may be an appreciable quantity.

SUMMARY AND CONCLUSION

Temperature determinations of skin surfaces, deep skin, subcutaneous tissue, muscles and blood in the precubital veins were made on normal individuals and on normally afebrile patients undergoing artificial fever treatments. Such observations were made under normal circumstances, at various stages of the fever production by 1, intravenous vaccine; 2, the "blanket pack" method alone, and 3, the "blanket pack" preceded by heating in an electric light cabinet. Observations were likewise made on such individuals during cooling by surface sponging and by inspiring iced air. Individuals with normal temperature were likewise studied under the influence of cooled air inhalation.

The recognized gradients between the deep tissue and surface skin were observed with the exception that occasionally the skin was warmer than the subcutaneous tissue under normal circumstances. This phenomenon generally occurred during artificial fever with the "blanket pack" method. The behavior of the intravenous blood temperature under various circumstances leads to the conclusion that the blood may serve as an important cooling agent to the general body tissues, losing more heat in the respiratory tract than has been believed heretofore.

REFERENCES

- (1) BECQUEREL, E. AND BRESCHET. C. R. de l'Acad. d. Sc. 1: 28, 1835; Ann. Sc. Nat. 2: Ser. 3: 257, 1835, 1839; 4: 243, 1836; 7: 94, 1839; (Cit.) 9: 271, 1841.
- (2) LEFEVRE, J. Chaleur Animale Paris, 1911. (Cit).
- (3) ZONDEK, B. Münch. Med. Wehnschr. 66: 1315, 1379, 1919; 67: 255, 810, 1041, 1920.
- (4) BAZETT, H. C. AND B. McGLONE. This Journal 76: 222, 1926; 82: 415, 1927.
- (5) FOGED, J. Skand Arch. Physiol. 59: 109, 1930.
- (6) WRIGHT, I. S. AND H. J. JOHNSON. Proc. Soc. Exper. Biol and Med. 30: 759, 1933.
- (7) BERNARD, C. L. Compt. R. Acad. d. Sc. 40: 331, 561, 1855.
- (8) LIEBIG, G. Über die Temperaturunterschiede des Venosen und Arteriellen Blutes. Giessen Thesis, 1853. (Cit).
- (9) COLIN, M. Arch. Gen. de Med. 6th Ser. 2: 45, 1865.
- (10) BAZETT, H. C. Physiol. Rev. 7: 531, 1927.
- (11) DEIGHTON, T. Physiol. Rev. 13: 427, 1933.
- (12) EPSTEIN, N. AND M. COHEN. J. A. M. A. 104: 883, 1935.
- (13) TIGERSTEDT, R. A text book of human physiology, p. 403. Transl. from 3rd German ed. by J. R. MURLIN. D. Appleton & Co., New York and London, 1906.
- (14) BARACH, A. L. Arch. Int. Med. 37: 186, 1926.

INDEX

- ABBOTT, E. and A. C. IVY.** The effect of lactation and exercise on the rate of involution of the uterus in the rat, 487.
- Absorption, intestinal, anoxemia and, 309.
- of glucose and sucrose, relative rates of, 257.
- Acetylcholine, blood sugar and amino acids, 542.
- , response of spleen to, 701.
- Action and excitability in mammalian A fibers, 113.
- Activation of blood coagulation, 587.
- Adolescence, pre-, exercise and respiration in, 577.
- ADOLPH, E. F.** Control of urine formation in the frog by the renal circulation, 366.
- Adrenal cortex, relation of, to vitamin C, 553.
- cortical extracts, bioassay of, 678.
- Adrenalectomized dog, blood sugar of, 13.
- nephrectomized rat, survival of, 200.
- ALLEN, E., A. W. DIDDLE, T. H. BURFORD and W. U. GARDNER.** Ovarian hormone threshold for experimental menstruation in monkeys, 381.
- AMBERSON, W. R.** See **STANBURY, WARWEG and AMBERSON**, 230.
- Amino acids, effect of acetylcholine on blood sugar and, 542.
- Anaerobic glycolysis in tissues from polyneuritic chicks, 151.
- Androstendion, relation of, to protein metabolism, 642.
- Anoxemia and intestinal absorption, 309.
- Artery, circumflex coronary, blood flow in, 271.
- Atrophy, simple disuse, in monkey, 626.
- AUSTIN, J. H.** See **SUNDERMAN and AUSTIN**, 474.
- BACHRACH, W. H., W. B. BRADLEY and A. C. IVY.** Effect of epinephrine on glucose excretion in fasted depancreatized dogs, 203.
- BALDES, E. J.** See **ESSEX, HERRICK, BALDES and MANN**, 271.
- BARTLEY, S. H.** A comparison of the electrogram of the optic cortex with that of the retina, 338.
- BARTZ, J. P. and F. O. SCHMITT.** Carotene and associated pigments in medullated nerve, 280.
- BAZETT, H. C.** See **BURTON and BAZETT**, 36.
- BERGMAN, H. C.** See **DRURY, BERGMAN and GREELEY**, 323.
- BERNHART, F.** See **Hemingway, COLLINS and BERNHART**, 102.
- Bile acids, blood clearance and renal excretion of, after injection of cholic and desoxycholic acids, 665.
- , fecal fat in absence of, 525.
- Bioassay of adrenal cortical extracts, 678.
- BISCHOFF, F.** Histone combinations of the protein hormones, 182.
- BISHOP, G. H. and J. J. BRONFENBRENNER.** The site of action of botulinus toxin, 393.
- and **J. O'LEARY.** Components of the electrical response of the optic cortex of the rabbit, 292.
- BLAIR, E. A. and J. ERLANGER.** Temporal summation in peripheral nerve fibers, 355.
- BLALOCK, A. and M. F. MASON.** Observations on the blood flow and gaseous metabolism of the liver of unanesthetized dogs, 328.
- Blood, circulating, cooling effect of, 708.
- clearance and renal excretion of bile acids after injection of cholic and desoxycholic acids, 665.
- coagulation, activation of, 587.

- Blood coagulation, certain sulfur compounds and, 92.
- , distribution of glucose in, 335.
- flow and gaseous metabolism of liver, 328.
- — in circumflex coronary artery, 271.
- plasma dilution after saline injection, 102.
- pressure variations, spinal vasomotor reflexes and, 619.
- serum volume, measurement of, 474.
- specific gravity during excitement, 111.
- sugar and amino acids, effect of acetylcholine on, 542.
- — of adrenalectomized dog, 13.
- Bone marrow of rat, nucleated cells in, 662
- Botulinus toxin, site of action of, 393.
- BOUCKAERT, J. J. See HEYMANS, BOUCKAERT, FARBER and HSU, 619.
- BOZLER, E. The mechanism of the inhibitory action of vasodilator nerves, 457.
- BRADLEY, W. B. See BACHRACH, BRADLEY and IVY, 203.
- Brain potentials, components of, 292.
- Breathing, central mechanism controlling, 423.
- BRICKER, J. See GESELL, BRICKER and MAGEE, 423.
- BROH-KAHN, R. H. See MIRSKY and BROH-KAHN, 6.
- BRONFENBRENNER, J. J. See BISHOP and BRONFENBRENNER, 393.
- BRONK, D. W., L. K. FERGUSON, R. MARGARIA and D. Y. SOLANDT. The activity of the cardiac sympathetic centers, 237.
- BUNTING, R. W. See WHITE and BUNTING, 529.
- BURFORD, T. H. See ALLEN, DIDDLE, BURFORD and GARDNER, 381.
- BURTON, A. C. and H. C. BAZETT. A study of the average temperature of the tissues, of the exchanges of heat and vasomotor responses in man by means of a bath calorimeter, 36.
- BUSSABARGER, R. A., and F. T. JUNG. Dietary and hematologic studies after gastrectomy in the rat, 59.
- CALORIGENIC action of vitamin D, rôle of thyroid in, 1.
- Calorimeter, bath, heat exchange and vasomotor responses in, 36.
- Carbohydrate metabolism, hyperthyroidism and, 6.
- Carbon dioxide and visual intensity discrimination, 75.
- Cardiac sympathetic centers, activity of, 237.
- Cardiovascular activity, cerebral cortical influence on, 411.
- Carotene and other pigments in medullated nerve, 280.
- CARTLAND, G. F. and M. H. KUIZENGA. The bioassay of adrenal cortical extracts, 678.
- Cells, nucleated, in bone marrow of rat, 662.
- Cerebellar stimulation, spinal path for responses to, 267.
- Cerebellum, electrical stimulation of interior of, in decerebrate cat, 261.
- Cerebral cortical influence on cardiovascular activity, 411.
- CHOR, H. and R. E. DOLKART. A study of "simple disuse atrophy" in the monkey, 626.
- Chronaxie, the, strychnine and, 638.
- Circulatory control of urine formation, 366.
- Coagulation, blood, activation of, 587.
- of blood, certain sulfur compounds and, 92.
- Cochlear response, electrical, of cats, 24.
- Coeliac ganglion cells of rabbits, functional behavior of, 514.
- COLLINS, D. A. See HEMINGWAY, COLLINS and BERNHART, 102.
- Copper, augmented action of pituitary extracts by, 68.
- Coronary artery, circumflex, blood flow in, 271.
- Cortex, adrenal, relation of, to vitamin C, 553.
- Cortical extracts, adrenal, bioassay of, 678.

- Cortical influence on cardiovascular activity, 411.
- CRAMPTON, C. B. See SCHNEIDER and CRAMPTON, 577.
- CRANDALL, L. A., JR. and G. M. ROBERTS. Increased water exchange following Eck fistula in dogs, 318.
- Cretinism, experimental, pituitary in, 518.
- Crossed respiratory impulses to phrenic, 495.
- DAVIS, B. L., JR. and J. M. LUCK. The effect of acetylcholine and other constituents of the suprarenal gland upon blood sugar and amino acids, 542.
- Dental caries, chemical composition of saliva and, 529.
- Deuterium as indicator of fecal fat in absence of bile, 525.
- DEUTSCH, H., C. I. REED and H. C. STRUCK. The rôle of the thyroid in the calorogenic action of vitamin D, 1.
- Diabetes, pancreatic, relation of pancreatic juice to, 160.
- DIDDLE, A. W. See ALLEN, DIDDLE, BURFORD and GARDNER, 381.
- Diet, effect of raw pancreas in, after pancreatectomy, 166.
- DOLKART, R. E. See CHOR and DOLKART, 626.
- DRAGSTEDT, L. R., J. VAN PROHASKA and H. P. HARMS. Observations on a substance in pancreas (a fat metabolizing hormone) which permits survival and prevents liver changes in depancreatized dogs, 175.
- See HARMS, VAN PROHASKA and DRAGSTEDT, 160.
- See VAN PROHASKA, DRAGSTEDT and HARMS, 166.
- DRURY, D. R., H. C. BERGMAN and P. O. GREELEY. The glucose utilization of phloridzinised dogs after hepatectomy, 323.
- Duodenal secretions and experimental jejunal ulcer, 79.
- EAR. See Cochlear.
- Eck fistula, increased water exchange after, 318.
- Electrical cochlear response of cats, 24.
- response potentials from skin, 189.
- stimulation of interior of cerebellum in decerebrate cat, 261.
- Electrograms of optic cortex and retina, 338.
- ELVEHJEM, C. A. See SHERMAN and ELVEHJEM, 142, 151.
- Embryo heart, transplant of sino-atrium to conus in, 313.
- Endocrine complex, female, eosinophil leucocytes in, 250.
- Environmental conditions, germinal response to, 285.
- Eosinophil leucocytes in female endocrine complex, 250.
- Epinephrine, effect of, on glucose excretion, 203.
- ERLANGER, J. See BLAIR and ERLANGER, 355.
- ESSEX, H. E., J. F. HERRICK, E. J. BALDES and F. C. MANN. Blood flow in the circumflex branch of the left coronary artery of the dog, 271.
- EVANS, E. I., S. SZUREK and R. KERN. The tetany of oestrus in the parathyroidectomized dog, 405.
- Excretion, glucose, effect of epinephrine on, 203.
- of urea in dog, glomerular filtration and, 206.
- Exercise and respiration in pre-adolescent boys, 577.
- , effect of lactation and, on involution of uterus, 487.
- , urea clearance and proteinuria during, 658.
- FAGIN, J. and S. R. M. REYNOLDS. The endometrial vascular bed in relation to rhythmic uterine motility, with a consideration of the functions of the intermittent contractions of oestrus, 86.
- FARBER, S. See HEYMANS, BOUCKAERT, FARBER and HSU, 619.
- FARRAR, G. E., JR. The concentration of nucleated cells in the bone marrow of the albino rat, 662.
- FARRELL, J. I. and Y. LYMAN. The effect of occlusion of the outflow of

- prostatic secretion on the prostate gland, 559.
- Fat, fecal, in absence of bile, 525.
- metabolizing hormone of pancreas, 175.
- FAZEKAS, J. F. See GOLDFARB, FAZEKAS and HIMWICH, 631.
- Fecal fat in absence of bile, 525.
- FERGUSON, J., A. C. IVY and H. GREENGARD. Observations on the response of the spleen to the intravenous injection of certain secretin preparations, acetyl choline and histamine, 701.
- FERGUSON, J. H. An experimental analysis of coagulant activation, 587.
- FERGUSON, L. K. See BRONK, FERGUSON, MARGARIA and SOLANDT, 237.
- Fever, artificial, depth temperatures in, 708.
- FEVOLD, H. L., F. L. HISAW and R. GREEP. Augmentation of the gonad stimulating action of pituitary extracts by inorganic substances, particularly copper salts, 68.
- FISCHER, E. The action of a single vagal volley on the heart of the eel and the turtle, 596.
- FISHMAN, D. See NICE and FISHMAN, 111.
- FORBES, T. W. Skin potential and impedance responses with recurring shock stimulation, 189.
- See FOWLER and FORBES, 24.
- FOWLER, E. P., JR. and T. W. FORBES. Depression in order of frequency of the electrical cochlear response of cats, 24.
- Fructose, utilization of, in mammals, 134.
- GARDNER, W. U. See ALLEN, DIDDLE, BURFORD and GARDNER, 381.
- GASSER, H. S. and H. GRUNDFEST. Action and excitability in mammalian A fibers, 113.
- Gastrectomy and vagotomy, gastric acidity after, 533.
- in the rat, effects of, 59.
- Gastric acidity after gastrectomy and vagotomy, 533.
- emptying time after acute hemorrhage, 226.
- GELHORN, E. The effectiveness of carbon dioxide in combating the changes in visual intensity discrimination produced by oxygen deficiency, 75.
- Germinal response to environmental conditions, 285.
- GESELL, R., J. BRICKER and C. MAGEE. Structural and functional organization of the central mechanism controlling breathing, 423.
- Glomerular filtration and urea excretion in dog, 206.
- Glucose and sucrose, relative rates of absorption of, 257.
- , distribution of, in blood, 335.
- excretion, effect of epinephrine on, 203.
- utilization after phlorhidzin and hepatectomy, 323.
- Glycolysis, anaerobic, in polyneuritic chick tissue, 151.
- , —, in tissues from polyneuritic chicks, 151.
- GOLDFARB, W., J. F. FAZEKAS and H. E. HIMWICH. The effect of methylene blue, cystine and cysteine on the metabolism of the intact animal, 631.
- Gonadotropic action of pituitary extracts augmented by copper, 168.
- GREELEY, P. O. See DRURY, BERGMAN and GREELEY, 323.
- GREEN, H. D. See HOFF and GREEN, 411.
- GREENGARD, H. See FERGUSON, IVY and GREENGARD, 701.
- GREEP, R. See FEVOLD, HISAW and GREEP, 68.
- GRIFFITHS, J. P. and E. T. WATERS. The utilization of fructose in the mammalian organism as shown by experiments on hepatectomized and eviscerated preparations, 134.
- GRUNDFEST, H. See GASSER and GRUNDFEST, 113.

- HAMILTON, J. W., JR.** See **KELLER, NOBLE** and **HAMILTON**, 467.
- HARE, W. K., H. W. MAGOUN** and **S. W. RANSON**. Electrical stimulation of the interior of the cerebellum in the decerebrate cat, 261.
- HARMS, H. P., J. VAN PROHASKA** and **L. R. DRAGSTEDT**. The relation of pancreatic juice to pancreatic diabetes, 160.
- See **DRAGSTEDT, VAN PROHASKA** and **HARMS**, 175.
- See **VAN PROHASKA, DRAGSTEDT** and **HARMS**, 166.
- HARTMAN, F. A.** See **LOCKWOOD, SWAN** and **HARTMAN**, 553.
- HAYS, H. W.** See **PARKINS, HAYS** and **SWINGLE**, 13.
- Heart, eel**, response of, to a single vagal volley, 596.
- , embryo, transplant of sino-atrium to conus in, 313.
- , *Limulus*, pacemaker mechanism in, 686.
- Heat exchange and vasomotor responses**, 36.
- HEINBECKER, P.** The potential analysis of a pacemaker mechanism in *Limulus polyphemus*, 686.
- HEMINGWAY, A., D. A. COLLINS** and **F. BERNHART**. A comparison of three methods of measuring plasma dilution after intravenous saline injection into normal anesthetized and functionally eviscerated dogs, 102.
- Hemorrhage, acute**, emptying time of stomach after, 226.
- Hepatectomy, effect of**, on utilization of fructose in mammals, 134.
- , glucose utilization after phloridzin and, 323.
- HERRICK, J. F.** See **ESSEX, HERRICK, BALDES** and **MANN**, 271.
- HEYMANS, C., J. J. BOUCKAERT, S. FARBER** and **F. Y. HSU**. Spinal vasomotor reflexes associated with variations in blood pressure, 619.
- HILL, F. C.** See **WILHELMJ, MCCARTHY** and **HILL**, 533.
- See **WILHELMJ, O'BRIEN, MCCARTHY** and **HILL**, 79.
- HIMWICH, H. E.** See **GOLDFARB, FAZEKAS** and **HIMWICH**, 631.
- HISAW, F. L.** See **FEVOLD, HISAW** and **GREEP**, 68.
- Histamine**, response of spleen to, 701.
- Histone combinations of protein hormones**, 182.
- HOFF, E. C. and H. D. GREEN**. Cardiovascular reactions induced by electrical stimulation of the cerebral cortex, 411.
- Hormone, fat metabolizing, of pancreas**, 175.
- , male, relation of, to protein metabolism, 642.
- , ovarian, in experimental menstruation, 381.
- Hormones, protein, histone combinations of**, 182.
- HSU, F. Y.** See **HEYMANS, BOUCKAERT, FARBER** and **HSU**, 619.
- HUNTER, W. S.** See **PROSSER** and **HUNTER**, 609.
- Hyperthyroidism and carbohydrate metabolism**, 6.
- Hypophysis, effects of separation of, from hypothalamus**, 467.
- Hypothalamus, anterior, rôle of, in temperature regulation**, 562.
- , effects of separation of hypophysis from, 467.
- INGERSOLL, E. H.** Functional behavior of coeliac ganglion cells of the rabbit, 514.
- , **H. W. MAGOUN** and **S. W. RANSON**. The spinal path for responses to cerebellar stimulation, 267.
- INGLE, D. J. and E. C. KENDALL**. Survival of the adrenalectomized nephrectomized rat, 200.
- Inhibitory action of vasodilator nerves**, 457.
- Insulin, protamine, intravenous administration of**, 453.
- Intestinal absorption, anoxemia and**, 309.
- Intravenous administration of protamine insulin**, 453.
- Ivy, A. C.** See **ABBOTT** and **Ivy**, 487.

- IVY, A. C. See BACHRACH, BRADLEY and IVY, 203.
 —. See FERGUSON, IVY and GREENGARD, 701.
- J** EJUNAL ulcer, experimental, duodenal secretions and, 79.
- JUNG, F. T. See BUSSABARGER and JUNG, 59.
- K** ATZ, H. L. See NICE and KATZ, 571.
 KELLER, A. D., W. NOBLE and J. W. HAMILTON, JR. Effects of anatomical separation of the hypophysis from the hypothalamus in the dog, 467.
- KENDALL, E. C. See INGLE and KENDALL, 200.
- KERN, R. See EVANS, SZUREK and KERN, 405.
- KNOEFEL, P. K. Strychnine and the chronaxie, 638.
- KOCHAKIAN, C. D. and J. R. MURLIN. The relationship of the synthetic male hormone, androstendion, to the protein and energy metabolism of castrate dogs, and the protein metabolism of a normal dog, 642.
- KOSTER, H. See SHAPIRO, KOSTER, RITTENBERG and SCHOENHEIMER, 525.
- KRAATZ, C. P. A possible rôle of the eosinophil leucocytes in the endocrine complex of the female rat, 250.
- KUIZENGA, M. H. See CARTLAND and KUIZENGA, 678.
- L** ACTATION and exercise, effect of, on involution of uterus, 487.
 Leucocytes, eosinophil, in female endocrine complex, 250.
 Leucopenia, emotional, in rabbits, 571.
- LICHTMAN, S. S. The blood clearance and renal excretion of bile acids following the intravenous injection of cholic and desoxycholic acids, 665.
- LIGHT, A. B. and C. R. WARREN. Urea clearance and proteinuria during exercise, 658.
- Limulus polyphemus, potential analysis of pacemaker mechanism in, 686.
- Liver, blood flow and gaseous metabolism of, 328.
 — degeneration in depancreatized dog, relation of pancreatic juice to, 166.
 — removal, glucose utilization after phloridzin and, 323.
- LOCKWOOD, J. E., D. R. SWAN and F. A. HARTMAN. A further study of the relation of the adrenal cortex to vitamin C, 553.
- LONGWELL, B. B. and A. RAVIN. The effect of intravenous administration of protamine insulin, 453.
- LUCK, J. M. See DAVIS and LUCK, 542.
- LYMAN, Y. See FARRELL and LYMAN, 559.
- M** CCARTHY, H. H. See WILHELMJ, McCARTHY and HILL, 533.
 —. See WILHELMJ, O'BRIEN, McCARTHY and HILL, 79.
- MAGEE, C. See GESELL, BRICKER and MAGEE, 423.
- MAGOUN, H. W. See HARE, MAGOUN and RANSON, 261.
 —. See INGERSOLL, MAGOUN and RANSON, 267.
- Male hormone, relation of, to protein metabolism, 642.
- MANN, F. C. See ESSEX, HERRICK, BALDES and MANN, 271.
- MARGARIA, R. See BRONK, FERGUSON, MARGARIA and SOLANDT, 237.
- MASON, M. F. See BLALOCK and MASON, 328.
- MEDES, G. See STERNER and MEDES, 92.
- Menstruation, experimental, ovarian hormone in, 381.
- Metabolism, basal, NaF administration and, 155.
 —, carbohydrate, hyperthyroidism and, 6.
 —, effect of methylene blue, cystine and cysteine on, 631.
 —, gaseous, of liver, blood flow and, 328.
 —, protein, relation of male hormone to, 642.
- MIRSKY, I. A. and R. H. BROH-KAHN. The effect of experimental hyper-

- thyroidism on carbohydrate metabolism, 6.
- Movements, vertical, respiratory reactions upon, 349.
- MURLIN, J. R. See KOCHAKIAN and MURLIN, 642.
- Muscle atrophy, simple disuse, 626.
- NAF** administration and basal metabolism, 155.
- Nephrectomized rat, adrenalectomized-, survival of, 200.
- Nerve cells, coeliac ganglion, of rabbit, functional behavior of, 514.
- centers, cardiac sympathetic, activity of, 237.
- fibers, action and excitability in, 113.
- —, temporal summation in, 355.
- , medullated, carotene and other pigments in, 280.
- , phrenic, crossed respiratory impulses to, 495.
- Nerves, vasodilator, inhibitory action of, 457.
- NEUWIRTH, I. The distribution of glucose in blood, 335.
- NICE, L. B. and D. FISHMAN. The specific gravity of the blood of pigeons in the quiet state and during emotional excitement, 111.
- and H. L. KATZ. Emotional leucopenia in rabbits, 571.
- NOBLE, W. See KELLER, NOBLE and HAMILTON, 467.
- NORTHUP, D. See VAN LIERE, SLEETH and NORTHUP, 226.
- Nucleated cells in bone marrow of rat, 662.
- O'BRIEN, F. T. See WILHELMJ, O'BRIEN, MCCARTHY and HILL, 79.
- Oestrin production, vaginal and uterine grafts as indicators of, 672.
- Oestrus after parathyroidectomy, tetany of, 405.
- , functions of intermittent contractions of, 86.
- OGLE, C. L. Germinal response (in male mice) to environmental conditions, 285.
- O'LEARY, J. See BISHOP and O'LEARY, 292.
- Optic cortex and retina, electrograms of, 338.
- —, responses of, to stimulation of optic nerve, 292.
- ORTIZ, T. See ROSENBLUETH and ORTIZ, 495.
- Ovarian hormone in experimental menstruation, 381.
- Oxygen deficiency and CO₂ in visual intensity discrimination, 75.
- PACEMAKER** mechanism in *Limulus*, potential analysis of, 686.
- PAFF, G. H. Transplantation of sinoatrium to conus in the embryonic heart in vitro, 313.
- Pancreas, fat metabolizing hormone of, 175.
- Pancreatic juice, relation of, to liver degeneration in depancreatized dog, 166.
- —, relation of, to pancreatic diabetes, 160.
- Parathyroidectomy, tetany of oestrus after, 405.
- PARKINS, W. M., H. W. HAYS and W. W. SWINGLE. A study of the blood sugar of the adrenalectomized dog, 13.
- PARTINGTON, P. P. The production of sympathin in response to physiological stimuli in the unanesthetized animal, 55.
- PFEIFFER, C. A. Vaginal and uterine grafts in the rat as indicators of the production of oestrin, 672.
- PHILLIPS, P. H. Further studies on the effects of NaF administration upon the basal metabolic rate of experimental animals, 155.
- Phloridzin and hepatectomy, glucose utilization after, 323.
- Phrenic, crossed respiratory impulses to, 495.
- Pituitary extracts, augmented action of, by copper, 68.
- in experimental cretinism, 518.
- Plasma dilution after intravenous saline injection, 102.
- Plasmapheresis, total, 230.

- Polyneuritic chick tissue, anaerobic glycolysis in, 151.
- Polyneuritis, vitamin B₁ and pyruvic acid oxidation in, 142.
- PROSSER, C. L. and W. S. HUNTER. The extinction of startle responses and spinal reflexes in the white rat, 609.
- Prostatic secretion, effect of occlusion of outflow of, 559.
- Protamine insulin, intravenous administration of, 453.
- Protein hormones, histone combinations of, 182.
- metabolism, relation of male hormone to, 642.
- Proteinuria, urea clearance and, during exercise, 658.
- Pyruvic acid oxidation in polyneuritis, vitamin B₁ and, 142.
- R**ANSON, S. W. See HARE, MAGOUN and RANSON, 261.
- See INGERSOLL, MAGOUN and RANSON, 267.
- See TEAGUE and RANSON, 562.
- RAVIN, A. See LONGWELL and RAVIN, 453.
- REED, C. I. See DEUTSCH, REED and STRUCK, 1.
- Reflexes, extinction of responses and, 609.
- , spinal vasomotor, and variations in blood pressure, 619.
- Renal circulation, control of urine formation by, 366.
- excretion of bile acids, blood clearance and, after injection of cholic and desoxycholic acids, 665.
- Respiration, exercise and, in pre-adolescent boys, 577.
- Respiratory impulses, crossed, to phrenic, 495.
- mechanism, central, 423.
- reactions upon vertical movements, 349.
- Responses and reflexes, extinction of, 609.
- Retina, electrograms of optic cortex and, 338.
- REYNOLDS, S. R. M. See FAGIN and REYNOLDS, 86.
- RITTENBERG, D. See SHAPIRO, KOSTER, RITTENBERG and SCHOENHEIMER, 525.
- ROBERTS, A. C. A study of the speed of absorption following the ingestion of glucose and of sucrose, 257.
- ROBERTS, G. M. See CRANDALL and ROBERTS, 318.
- ROSENBLUETH, A. and T. ORTIZ. The crossed respiratory impulses to the phrenic, 495.
- S**ALIVA, resting, chemical composition of, 529.
- SAMPSON, J. J. Study of depth temperatures in artificial fevers and cooling air chambers with especial reference to cooling effect of the circulating blood, 708.
- SCHMITT, F. O. See BARTZ and SCHMITT, 280.
- SCHNEIDER, E. C. and C. B. CRAMPTON. The respiratory responses of pre-adolescent boys to muscular activity, 577.
- SCHOENHEIMER, R. See SHAPIRO, KOSTER, RITTENBERG and SCHOENHEIMER, 525.
- Secretin preparations, response of spleen to, 701.
- Secretions, duodenal, and experimental jejunal ulcer, 79.
- Serum volume, measurement of, 474.†
- SHANNON, J. A. Glomerular filtration and urea excretion in relation to urine flow in the dog, 206.
- SHAPIRO, A., H. KOSTER, D. RITTENBERG and R. SCHOENHEIMER. The origin of fecal fat in the absence of bile, studied with deuterium as an indicator, 525.
- SHERMAN, W. C. and C. A. ELVEHJEM. A study of anaerobic glycolysis in tissues from polyneuritic chicks, 151.
- — —. In vitro action of crystalline vitamin B₁ on pyruvic acid metabolism in tissues from polyneuritic chicks, 142.
- Skin, electrical response potentials from, 189.
- SLEETH, C. K. See VAN LIERE and SLEETH, 309.

- SLEETH, C. K. See VAN LIERE, SLEETH and NORTHUP, 226.
- SOLANDT, D. Y. See BRONK, FERGUSON, MARGARIA and SOLANDT, 237.
- SPIEGEL, E. A. Respiratory reactions upon vertical movements, 349.
- Spinal path for responses to cerebellar stimulation, 267.
- vasomotor reflexes and variations in blood pressure, 619.
- Spleen, response of, to secretin preparations, 701.
- STANBURY, J. B., E. WARWEG and W. R. AMBERSON. Total plasmapheresis, 230.
- STERNER, J. H. and G. MEDES. The effect of certain sulfur compounds on the coagulation of blood, 92.
- Stomach, emptying time of, after acute hemorrhage, 226.
- See Gastric.
- STRUCK, H. C. See DEUTSCH, REED and STRUCK, 1.
- Strychnine and the chronaxie, 638.
- Sucrose, glucose and, relative rates of absorption of, 257.
- Sugar of blood of adrenalectomized dog, 13.
- Sulfur compounds, certain, and blood coagulation, 92.
- Summation, temporal, in peripheral nerve fibers, 355.
- SUNDERMAN, F. W. and J. H. AUSTIN. The measurement of serum volume, 474.
- Suprarenal, relation of, to blood sugar and amino acids, 542.
- SWAN, D. R. See LOCKWOOD, SWAN and HARTMAN, 553.
- SWINGLE, W. W. See PARKINS, HAYS and SWINGLE, 13.
- Sympathetic nerve centers, cardiac, activity of, 237.
- Sympathin, physiological liberation of, 55.
- SZUREK, S. See EVANS, SZUREK and KERN, 405.
- TEAGUE, R. S. and S. W. RANSON. The rôle of the anterior hypothalamus in temperature regulation, 562.
- Teeth. See Dental.
- Temperature regulation, rôle of anterior hypothalamus in, 562.
- Temperatures, depth, in man, 708.
- Temporal summation in peripheral nerve fibers, 355.
- Tetany of oestrus after parathyroidectomy, 405.
- Toxin, botulinus, site of action of, 393.
- Thyroid, rôle of, in calorogenic action of vitamin D, 1.
- Thyrotropic effect of pituitaries from cretin rats, 518.
- UREA clearance and proteinuria during exercise, 658.
- excretion, glomerular filtration and, in dog, 206.
- Urine formation, circulatory control of, 366.
- Uterine grafts, vaginal and, as indicators of oestrin production, 672.
- motility, local vascular changes and, 86.
- Uterus, effect of lactation and exercise on involution of, 487.
- VAGAL volley, single, response of eel heart to, 596.
- Vaginal and uterine grafts as indicators of oestrin production, 672.
- Vagotomy, gastric acidity after gastrectomy and, 533.
- VAN LIERE, E. J. and C. K. SLEETH. Absorption of sodium chloride from the small intestine at various degrees of anoxemia, 309.
- , C. K. SLEETH and D. NORTHUP. The effect of acute hemorrhage on the emptying time of the stomach, 226.
- VAN PROHASKA, J., L. R. DRAGSTEDT and H. P. HARMS. The relation of pancreatic juice to the fatty infiltration and degeneration of the liver in the depancreatized dog, 166.
- See DRAGSTEDT, VAN PROHASKA and HARMS, 175.
- See HARMS, VAN PROHASKA and DRAGSTEDT, 160.
- Vascular changes, local, and uterine motility, 86.

- Vasodilator nerves, inhibitory action of, 457.
- Vasomotor responses, exchange of heat and, 36.
- Visual intensity discrimination, carbon dioxide and, 75.
- Vitamin B₁ and pyruvic acid oxidation in polyneuritis, 142.
- Vitamin C, relation of adrenal cortex to, 553.
- Vitamin D, rôle of thyroid in calorigenic action of, 1.
- WARREN, C. R.** See **LIGHT** and **WARREN**, 658.
- WARWEG, E.** See **STANBURY**, **WARWEG** and **AMBERSON**, 230.
- Water exchange, increased, following Eck fistula, 318.
- WATERS, E. T.** See **GRIFFITHS** and **WATERS**, 134.
- WHITE, J.** and **R. W. BUNTING.** A comparison of the chemical composition of stimulated and resting saliva of caries-free and caries-susceptible children, 529.
- WILHELMJ, C. M., H. H. MCCARTHY** and **F. C. HILL.** Gastric acidity following partial gastrectomy and vagotomy, 533.
- , **F. T. O'BRIEN, H. H. MCCARTHY** and **F. C. HILL.** The rôle of the duodenal secretions in the prevention of experimental jejunal ulcer, 79.
- ZECKWER, I. T.** Thyrotropic effect of pituitaries from cretin rats, 518.

